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# ECOLOGICAL AND EVOLUTIONARY DYNAMICS IN NATURAL POPULATIONS OF CO-EXISTING SEXUAL AND ASEXUAL LINEAGES

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# ECOLOGICAL AND EVOLUTIONARY DYNAMICS IN NATURAL POPULATIONS OF CO-EXISTING SEXUAL AND ASEXUALL LINEAGES

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# Table of Contents

# Summary

Theory predicts that asexually reproducing organisms should have a two-fold reproductive advantage over their sexual counterparts, which invest half of their reproductive potential into male offspring. The implications are that the descendants of a single asexual mutant can potentially replace a coexisting sexual population within tens of generations. On the other hand, asexual lineages are expected to be short-lived on evolutionary time scales because they lack mechanisms that rapidly generate new genetic diversity and are thus limited in their ability to adapt to changing environments. In this thesis, I focused on understanding 1) the spatial and temporal scales at which ecological and evolutionary processes favouring sexual reproduction occur and 2) the evolutionary origins and diversity of asexual lineages. As a model system I used a freshwater snail *Potamopyrgus antipodarum* that is characterized by frequent coexistence between diploid sexual and polyploid asexual individuals in its native New Zealand. Previous research on the *P. antipodarum* system has established that negative frequency-dependent selection by parasites provides a selective advantage to rare (e.g., sexually produced) genotypes and almost certainly plays an important role in the maintenance of sex and the coexistence of sexual and asexual individuals.

To assess the extent to which asexual *P. antipodarum* actually realize the predicted two-fold reproductive advantage, I measured reproductive output of sexual families and multiple different asexual lineages in a natural lake population using experimental enclosures. I found that the reproductive output of several asexual lineages is as high as the best sexual families, which implies that sexuals do pay the full "cost of sex" relative to some asexual lineages and that the coexistence of sexual and asexual lineages in the populations where sexuals and asexuals coexist and where asexual lineage fitness is high must involve factors giving sexuals a short-term selective advantage.

To assess the variation in ploidy level and genome size among asexual lineages, which were presumed to be triploid and nearly all female, an extensive survey aimed at characterizing ploidy-level variation was performed at a large geographic scale. The widespread occurrence

and multiple origins of polyploid males and individuals with higher than triploid ploidy level have been found, and the results indicate that they are likely to be descended from asexual females. The survey also suggested the existence of extensive variation in genome size. These results highlight the importance of considering ploidy level variation while investigating the origins and diversity of asexual lineages.

I addressed the questions of how the diversity of asexual lineages is generated by examining the evolutionary history of both mitochondrial and nuclear genetic markers across 16 lakes across the native New Zealand range of the *P. antipodarum* While the nuclear data suggest that asexual lineages are usually derived from coexisting sexual populations within lakes, the mitochondrial data is largely discordant with this pattern. In particular, the presence of a very common and widespread mitochondrial haplotype strongly associated with asexuality and paired with many different nuclear backgrounds indicates it had spread in parthenogenetic population due to selective advantage, and implies a more complex scenario of origin of new asexual lineages.

Finally, I examined the spatial variation and temporal dynamics of the genetic structure in a mixed sexual/asexual *P. antipodarum* population featuring a cline in the intensity of parasitism between two time points over a four-year interval. In habitats where parasite pressure is high, the genetic structure of asexual population (identities and frequencies of clones) changed significantly, while in the habitat where parasite pressure is low, it did not. The genetic structure of the sexual population also remained stable. These results are consistent with the Red Queen hypothesis for the maintenance of sex, which predicts that parasite mediated selection should disproportionately affect asexual genotypes when they become common, and give advantage to rare genotypes, therefore maintaining sex.

Overall, my results emphasize the importance of linking ecological, genetic and phylogeographic concepts and approaches when evaluating the evolution of reproductive modes in nature.

# Zussamenfassung

Laut Theorie haben Organismen, die sich asexuell fortpflanzen, einen doppelten Vorteil gegenüber sich sexuell Vermehrenden, da diese die Hälfte ihres Reproduktionspotentials in Männchen investieren müssen. Dies führt dazu, dass die Nachkommen einer einzigen asexuellen Mutante eine koexistierende sexuelle Population innerhalb weniger Generationen ersetzen könnten. Andererseits, über einen evolutionären Zeitraum gesehen, sollten asexuelle Fortpflanzungslinien kurzlebiger sein als sexuelle Linien. Asexuelle Linien haben keine geeigneten Mechanismen zur schnellen Erzeugung genetischer Diversität, die zur Anpassung an sich ändernde Umweltbedingungen aber nötig ist.

In dieser Arbeit konzentriere ich mich 1) auf das Verständnis räumlicher und zeitlicher Dimensionen, in denen ökologische und evolutionäre Prozesse die sexuelle Reproduktion favorisieren und 2) auf das Verständnis des evolutionären Ursprungs und der genetischen Diversität asexueller Linien. Als Modellsystem diente mir die Süsswasserschnecke *Potamopyrgus antipodarum*, da diese in ihrer Heimat Neuseeland häufig in gemischten Populationen aus diploiden sexuellen und polyploiden asexuellen Individuen vorkommt. Frühere Forschungen an *P. antipodarum* haben gezeigt, dass negativ frequenzabhängige Selektion durch Parasiten einen selektiven Vorteil für seltene (z.B. sexuell produzierte) Genotypen bietet und zweifelsohne sowohl eine wichtige Rolle in der Erhaltung sexueller Fortpflanzung als auch in der Koexistenz von asexueller und sexueller Fortpflanzung spielt.

Um das Ausmass zu bestimmen, in welchem asexuelle *P. antipodarum* tatsächlich den theoretischen zweifachen Vorteil nutzen, habe ich die Reproduktionsleistung asexueller Linien mit der sexueller Familien verglichen, die in experimentellen Käfigen einem Freilandversuch in ihrer natürlichen Umgebung gehalten wurden. Dabei fand ich, dass die Reproduktionsleistung mehrerer asexueller Linien höher war als die der besten sexuellen Familien. Daraus ergibt sich, dass sexuelle Familien tatsächlich die doppelten Kosten für sexuelle Reproduktion zahlen. Um die Koexistenz von sexuellen und asexuellen Linien mit

hoher Fitness innerhalb einer Population erklären zu können, müssen sexuelle Familien einen kurzfristigen selektiven Vorteil haben.

Um die Variabilität im Ploidiegrad und in der Genomgrösse zwischen asexuellen Linien bestimmen zu können, habe ich eine umfassende Erhebung über einen grossen geographischen Raum durchgeführt. Man geht davon aus, dass asexuelle Linien triploid sind und fast ausschliesslich aus Weibchen bestehen. Die Studie hat gezeigt, dass polyploide Männchen und Individuen mit höheren Ploidiegraden als Triploidie weiträumig verbreitet und multiplen Ursprungs sind. Dies deutet darauf hin, dass sie wahrscheinlich von asexuellen Weibchen abstammen. Ich konnte ebenfalls zeigen, dass die Variabilität in der Genomgrösse erheblich sein kann. Diese Ergebnisse betonen die Wichtigkeit, bei der Untersuchung des Ursprungs und der genetischen Diversität von asexuellen Linien die Variabilität im Ploidiegrad zu beachten.

Weiterhin untersuchte ich die Frage, wie die genetische Diversität asexueller Linien generiert wird, indem ich die evolutionäre Geschichte von sowohl mitochondrialen als auch nukleären genetischen Markern in 16 über das gesamte Verbreitungsgebiet von *P. antipodarum* in Neuseeland verteilten Seen bestimmt habe. Die nukleären Daten liessen vermuten, dass innerhalb eines Sees im Allgemeinen asexuelle Linien von koexistierenden sexuellen Populationen abstammen, während die mitochondrialen Daten grösstenteils davon abweichende Ergebnisse lieferten. Speziell das Vorhandensein eines sehr häufig und weitverbreitet vorkommenden, mitochodrialen Haplotyps, der stark mit Asexualität assoziert war und in vielen verschiedenen nuklären genetischen Hintergründen vorkam, lässt vermuten, dass er sich in parthenogenetischen Populationen durch einen selektiven Vorteil ausbreiten konnte. Dies impliziert ein wesentlich komplexeres Szenario der Herkunft neuer asexueller Linien.

Schliesslich habe ich noch die geographische und zeitliche Dynamik der genetischen Struktur in einer gemischten asexuellen/sexuellen Population von *P. antipodarum* untersucht, die einen graduellen Verlauf des Parasitierungsgrades über ein Intervall von vier Jahren aufwies. Während sich in Habitaten mit hohem Parasitendruck die genetische Struktur der asexuellen Population (Identität und Häufigkeit von klonalen Linien) signifikant veränderte, konnte dies in Habitaten mit geringem Parasitendruck nicht nachgewiesen werden. Die genetische

Struktur der sexuellen Population blieb ebenfalls stabil. Diese Ergebnisse sind konsistent mit der „Red Queen“ Hypothese zur Erhaltung von sexueller Fortpflanzung. Sie besagt, dass parasitenabhängige Selektion asexuelle Genotypen unverhältnismässig stark beeinflusst, sobald diese häufig werden. Seltene Genotypen hingegen werden bevorteilt, weshalb die sexuelle Fortpflanzung persistieren kann.

Abschliessend fasse ich zusammen, dass es eminent wichtig ist in Studien über die Evolution von Reproduktionsmodi in der Natur ökologische, genetische und phylogeographische Konzepte und Herangehensweisen zu vereinen.

# Introduction

## *Why is sexual reproduction so prevalent in nature?*

Sexual reproduction is a predominant reproductive mode among eukaryotes. However, the widespread occurrence of this reproductive mode calls for an evolutionary explanation of its short term benefits (Williams 1975; Maynard Smith 1978; Bell 1982), because when compared to asexual reproduction, sexual reproduction seems too costly to be common. Because only females directly produce offspring, a sexual lineage with a 1:1 sex ratio will have half of the rate of daughter production compared to an asexual lineage (assuming that sexual and asexual females produce the same number of offspring), which does not use resources on sons (the two-fold cost of sex, Maynard Smith 1978). Therefore, a mutation that causes a female in a sexual population to reproduce parthenogenetically will spread quickly through her descendants, and it is predicted that an asexual lineage can overtake a sexual population of $10^6$ individuals in less than 50 generations (Lively 1996).

The two-fold cost of sex exists under the assumption that all else is equal between sexual and asexual females (Maynard Smith 1978). The maintenance of sex would not be problematic if asexual females produced half the amount of offspring of sexual females. This theoretical two-fold advantage of asexuals is unlikely to be exactly accurate in nature, because the extent of the asexual advantage depends on other factors potentially influencing the outcome of competition between sexuals and asexuals. These factors include both further disadvantages of sexual reproduction, which make the anticipated costs of sexual reproduction higher than two fold, and also disadvantages or constraints of asexual reproduction which can reduce the baseline two-fold cost (reviewed in: Lewis 1987; Meirmans et al. 2012, see Table 1 for the overview). Given the complexity of factors affecting fitness of both sexual and asexual individuals, in studies of natural systems the most direct assessment of fitness of sexual and asexual lineages is of key importance, and should be a pre-requisite for studies concerning the maintenance of sex.

Table 1. Overview of costs of sexual reproduction, that can modify the baseline 'two fold' cost and hence the outcome of competition between sexual and asexual lineages (reviewed in: Lewis 1987; Hartfield and Keightley 2012; Meirmans et al. 2012).

| The additional costs of sex | Clarification/example | reference |
|---|---|---|
| Cost of meiosis | Genetic contribution of each parent to offspring is 50% (equivalent to two-fold cost for dioecious species) | (Williams 1975) |
| Cost of sexual selection | Expression of costly secondary sexual traits or behaviours | (Lewis 1987; Crow 1994, 1999) |
| Cost of sharing a genome | potential for genetic conflict when female genomes contain the genetic information for producing fit males | (Lewis 1987; Rice 2002) |
| Cost of mating | time and energy invested in mate searching, courtship and copulation and risks associated with these activities | (Bell 1982; Lewis 1987) |
| Cost of inbreeding, outbreeding and hybridization | inbreeding depression; breaking up advantageous associations between genes | (Lynch 1984) |
| Cost of sexually transmitted diseases and selfish genetic elements | sexually transmitted diseases, genetic parasites e.g. transposons; reproductive distortion (meiotic drive, *Wolbachia* endosymbionts) | (Crow 1994, 1999) |
| Biochemical costs and cost of having meiotic machinery | Applicable to microorganisms | (Lewis 1983) |
| The additional costs of asex /factors reducing cost of sex | | |
| Developmental or genetic constraints on the evolution of asexuality | Can make the cost of sex inapplicable (like e.g. in mammals), or cause reduced fitness in asexuals | (reviewed in: Engelstadter 2008) |
| Specific life history traits | e.g. female biased sex ratio and paternal care | (Maynard Smith 1978) |

## *Hypotheses explaining the maintenance of sex*

A number of hypotheses have been proposed to explain the maintenance of sex (reviewed in: Kondrashov 1993; West et al. 1999; Rice 2002; Otto 2009; Hartfield and Keightley 2012). Following the classification of Kondrashov (1993), the hypotheses have been often categorized as genetic/mutation-based or environmental/ecological, but these factors may also co-occur and interact.

Genetic models for the maintenance of sex initated from the idea that recombination helps to more efficiently eliminate deleterious mutations (e.g. Muller's ratchet, Muller 1964; Mutational deterministic hypothesis, Kondrashov 1988). More generally, the effect of recombination on the genome can be viewed as the force breaking apart selection interference between beneficial and deleterious mutations (Hill-Robertson effect, Hill and Robertson 1966; related models reviewed in: Otto 2009).

Ecological hypotheses are based on the concept that the advantage of sex lies in creating new gene combinations which accelerates the rate of adaptation to changing environment (e.g. Fisher-Muller hypothesis, Fisher 1930; Muller 1932; Frozen niche variation hypothesis, Vrijenhoek 1979; Tangled Bank hypothesis, Bell 1982). Apart from temporal and spatial variation of the physical environment, the interactions with other species (especially parasites), have been put forward as a likely source of selective forces favouring sexual reproduction (Red Queen hypothesis, Haldane 1949; Jaenike 1978; Hamilton 1980). The idea that time-lagged selection by co-evolving parasites against common host genotypes can provide a short-term advantage to sex has been supported both by theoretical models (for a recent review of Red Queen models see Lively 2010) and empirical studies (Antonovics and Ellstrand 1984; Ellstrand and Antonovics 1985; Lively 1987; Decaestecker 2007; Jokela et al. 2009; Wolinska and Spaak 2009).

However, the Red Queen hypothesis may also be limited in its generality. For once, it requires highly virulent or prevalent parasites to provide advantage to recombination(Howard and Lively 1994). Second, genetic variation and at least some degree of specificity for infectivity

and resistance needs to be present (Lively 1989; Agrawal and Lively 2002; Agrawal 2003). And finally, the main difficulty for the Red Queen hypothesis to provide a complete explanation for the maintenance of sex is that it selects for genetic diversity, and not sex as such (Lively and Howard 1994; King et al. 2011). When asexual population is as genetically diverse as the sexual one, parasite pressure is not enough to account for the two-fold cost of sex (Howard and Lively 1994; Lively and Howard 1994; Lythgoe 2000).

It has been proposed that mutational and ecological processes are likely to interact, which makes the conditions for the maintenance of sex broader (Howard and Lively 1994; West et al. 1999; Cooper et al. 2005;Park, 2010 #4476). For example, theory shows that the combined effects of mutation accumulation and parasite pressure can maintain sex under lower mutation rates and less virulent parasitism (Howard and Lively 1994). That is because parasites will force asexual lineages through population bottlenecks making the genetic drift stronger than selection. This can in turn accelerate the rate of accumulation of deleterious mutations and asexual lineage extinction. Also, synergistic interactions between infection and deleterious mutations can widen the conditions for the maintenance of sex, even when antagonistic epistasis between deleterious mutations is present (Park et al. 2010).

## *The study system and specific aims of this study*

The best model to study the maintenance of sex is a species where closely related sexual and asexual individuals coexist (Maynard Smith 1978; Bell 1982). In *Potamopyrgus antipodarum*, a prosobranch snail native to New Zealand freshwater lakes and rivers, obligately sexual, dioecious individuals coexist with asexual (parthenogenetic) females (Wallace 1985; Lively 1987; Wallace 1992). There is evidence for multiple independent transitions to asexuality (Dybdahl and Lively 1995a; Neiman and Lively 2004), and the asexuals were presumed to be triploid (Wallace 1992).To better understand the variation in distribution of asexual lineages, detect potential aberrartions in common ploidy patterns and to understand how ploidy level and genome size variation is connected to variation in mating system we conducted a survey of genome size and linked it with mitochondrial phylogeny (Chapter I, Neiman et al. 2011).

A previous population genetic study, using nuclear allozyme genotypes found that the asexual lineages are of local origin (Dybdahl and Lively 1995a). However, two geographically broader mitochondrial sequence-based phylogeographic studies (Neiman and Lively 2004; Neiman et al. 2011) suggested that while asexual *P. antipodarum* were often of local origin, some mitochondrial haplotypes were particularly common in asexuals and shared across many lakes. Because mitochondrial and nuclear genomes thus told different stories about the origin of asexual *P. antipodarum*, in Chapter II, we aimed to rigorously evaluate the presence and extent of mitonuclear incongruence and to gain some insight into its source. In particular, we used nuclear (SNP), mitochondrial and ploidy level data to ask whether phylogeographic and population genetic analyses of a multilocus nuclear dataset collected from a large ecologically and phylogenetically representative set of *P. antipodarum* would reveal evidence for widespread and common asexual lineages.

The asexual lineages of *P.antipodarum* have been found to be very diverse (Dybdahl and Lively 1995a; Fox et al. 1996). It has also been shown that asexual populations under strong parasite pressure are more diverse than populations with low parasite pressure (King et al. 2011). According to the model by Lively and Howard (1994) a diverse set of clones should replace a sexual population, as long as asexuals have a reproductive advantage, therefore a coexistence of diverse set of sexual lineages with a sexual population poses a challenge to our current understanding, and calls for comparison of fitness between sexual and asexual lineages. Previous study showed that sexual and asexual *P antipodarum* do not differ in key life history and reproductive traits (Jokela et al. 1997), which implies a cost of sex. More recent studies, however, suggest that the all-else-equal assumption may not hold for all *P. antipodarum* clones: Jokela et al. (2003) showed that clonal lineages from the same lake population varied dramatically in their reproductive success. Our aim in Chapter IV was to measure reproductive output of *P. antipodarum* snails in a field experiment, both to ask if there is cost of sex and to asses the variation in fitness among asexuals lineages. We also examined if the fitness of clones is habitat-dependent, the implication for the maintenance of sex being that the reproductive advantage of clones could be limited to certain habitats only.

The snails serve as an intermediate host to a number of trematode parasite species. The empirical evidence to date shows that parasites select against common *P. antipodarum* clones (Dybdahl and Lively 1995b; Lively and Dybdahl 2000; Jokela et al. 2009). Such selection on

the clonal population should lead to faster temporal dynamics of the clonal structure in habitats where selection by parasites is stronger. In Chapter III we tested this prediction by comparing the temporal changes in the genetic structures of clonal and sexual *P. antipodarum* in a population that features a cline in the intensity of parasitism over a four-year interval.

**Literature cited**


Agrawal, A., and C. M. Lively. 2002. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. Evol Ecol Res 4:79-90.

Agrawal, A. F., and C. M. Lively. 2003. Modelling infection as a two-step process combining gene-for-gene and matching alleles genetics. Proceedings of the Royal Society of London B, Biological Sciences 270:323-334.

Antonovics, J., and N. C. Ellstrand. 1984. Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. Evolution 38:103-115.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Univeristy of California Press, Berkeley (CA).

Cooper, T. F., R. E. Lenski, and S. F. Elena. 2005. Parasites and mutational load: an experimental test of a pluralistic theory for the evolution of sex. Proc. R. Soc. B-Biol. Sci. 272:311-317.

Crow, J. F. 1994. Advantages of sexual reproduction. Dev. Genet. 15:205-213.

Crow, J. F. 1999. The omnipresent process of sex. J Evolution Biol 12:1023-1025.

Decaestecker, E., S. Gaba, J. A. M. Raeymaekers, R. Stoks, L. Van Kerckhoeven, D. Ebert, and L. De Meester 2007. Host-parasite 'Red Queen' dynamics archived in pond sediment. Nature 450:870-873.

Dybdahl, M. F., and C. M. Lively. 1995a. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). J Evolution Biol 8:385-398.

Dybdahl, M. F., and C. M. Lively. 1995b. Host-parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). Proceedings of the Royal Society of London B, Biological Sciences 260:99-103.

Ellstrand, N. C., and J. Antonovics. 1985. Experimental studies on the evolutionary significance of sexual reproduction. II. A test of the density-dependent selection hypothesis. Evolution 39:657-666.

Engelstadter, J. 2008. Constraints on the evolution of asexual reproduction. Bioessays 30:1138-1150.

Fisher, R. A. 1930. Sexual reproduction and sexual selection. Pp. 121-145. The genetical theory of natural selection. Clarendon Press, Oxford.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (Potamopyrgus antipodarum). Evolution 50:1541-1548.

Haldane, J. B. S. 1949. Disease and evolution. La Ricerca Scientifica (Supplement) 19:68-76.

Hamilton, W. D. 1980. Sex Versus Non-Sex Versus Parasite. Oikos 35:282-290.

Hartfield, M., and P. D. Keightley. 2012. Current hypotheses for the evolution of sex and recombination. Integrative Zoology 7:192-209.
Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genet Res 8:269-294.
Howard, R., and C. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554-557.
Jaenike, J. 1978. An hypothesis to account for the maintenance of sex within populations. Evolutionary Theory 3:191-194.
Jokela, J., M. F. Dybdahl, and C. M. Lively. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. Am Nat 174:S43-S53.
Jokela, J., C. M. Lively, M. F. Dybdahl, and J. A. Fox. 1997. Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. Ecology 78:452-460.
King, K. C., J. Jokela, and C. M. Lively. 2011. Parasites, sex and clonal diversity in natural snail populations. Evolution 65:1474-1481.
Kondrashov, A. S. 1988. Deleterious Mutations and the Evolution of Sexual Reproduction. Nature 336:435-440.
Kondrashov, A. S. 1993. Classification of Hypotheses on the Advantage of Amphimixis. J Hered 84:372-387.
Lewis, W. M. 1983. Interruption of synthesis as a cost of sex in small organisms. Am Nat 121:825-834.
Lewis, W. M., Jr. 1987. The cost of sex. Pp. 33-57 *in* S. C. Stearns, ed. The evolution of sex and its consequences. Birkhäuser Verlag, Basel (Switzerland).
Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519-521.
Lively, C. M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. Evolution 43:1663-1671.
Lively, C. M. 1996. Host-parasite coevolution and sex - Do interactions between biological enemies maintain genetic variation and cross-fertilization? Bioscience 46:107-114.
Lively, C. M. 2010. A Review of Red Queen Models for the Persistence of Obligate Sexual Reproduction. J Hered 101:S13-S20.
Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405:679-681.
Lively, C. M., and R. S. Howard. 1994. Selection by parasites for clonal diversity and mixed mating. Philosophical Transactions of the Royal Society of London Series B 346:271-281.
Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes, and geographical parthenogenesis. Quarterly Review of Biology 59:257-290.
Lythgoe, K. A. 2000. The coevolution of parasites with host-acquired immunity and the evolution of sex. Evolution 54:1142-1156.
Maynard Smith, J. 1978. The evolution of sex. Cambridge Univeristy Press, Cambridge.
Meirmans, S., P. G. Meirmans, and L. R. Kirkendall. 2012. The Costs Of Sex: Facing Real-world Complexities. The Quarterly Review of Biology 87:19-40.
Muller, H. J. 1932. Some genetic aspects of sex. Am Nat 66:118-138.
Muller, H. J. 1964. The Relation of Recombination to Mutational Advance. Mutation Research 1:2-9.

Neiman, M., and C. M. Lively. 2004. Pleistocene glaciation is implicated in the phylogeographical structure of Potamopyrgus antipodarum, a New Zealand snail. Mol Ecol 13:3085-3098.
Neiman, M., D. Paczesniak, D. M. Soper, A. T. Baldwin, and G. Hehman. 2011. Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. Evolution 65:3202-3216.
Otto, S. P. 2009. The evolutionary enigma of sex. Am Nat 174:S1-S14.
Park, A. W., J. Jokela, and Y. Michalakis. 2010. Parasites and deleterious mutations: interactions influencing the evolutionary maintenance of sex. J Evolution Biol 23:1013-1023.
Rice, W. R. 2002. Experimental tests of the adaptive significance of sexual reproduction. Nature Review of Genetics 3:241-250.
Vrijenhoek, R. C. 1979. Factors Affecting Clonal Diversity and Coexistence. American Zoologist 19:787-797.
Wallace, C. 1985. On the Distribution of the Sexes of Potamopyrgus-Jenkinsi (Smith). J. Molluscan Stud. 51:290-296.
Wallace, C. 1992. Parthenogenesis, Sex and Chromosomes in Potamopyrgus. J. Molluscan Stud. 58:93-107.
West, S. A., C. M. Lively, and A. F. Read. 1999. A pluralist approach to sex and recombination. J Evolution Biol 12:1003-1012.
Williams, G. C. 1975. Sex and Evolution. Princeton University Press, Princeton (NJ).
Wolinska, J., and P. Spaak. 2009. The cost of being common: Evidence from natural *Daphnia* populations. Evolution 63:1893-1901.

# Chapter I

## Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages

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**Abstract**

Natural animal populations are rarely screened for ploidy-level variation at a scale that allows detection of potentially important aberrations of common ploidy patterns. This type of screening can be especially important for the many mixed sexual/asexual systems where sexuals are presumed to be dioecious diploids and asexuals are assumed to be triploid and all-female. For example, elevation of ploidy level above triploidy can be a source of genetic variation and raises the possibility of gene flow among ploidy levels and to asexual lineages. We used flow cytometry and mtDNA sequencing to characterize ploidy level and genome size in *Potamopyrgus antipodarum*, a New Zealand freshwater snail where obligate sexual (presumed diploid and dioecious) and obligate apomictic asexual (presumed triploid and nearly all female) individuals frequently coexist. We documented the widespread occurrence and multiple origins of polyploid males and individuals with >3x ploidy, and find that both are likely to be descended from asexual females. Our survey also suggested the existence of extensive variation in genome size. The discovery of widespread variation in ploidy level and genome size in such a well-studied system highlights the importance of broad, extensive, and ecologically representative sampling in uncovering ploidy level and genome size variation in natural populations.

**Introduction**

Asexual animals and plants are usually polyploid, and polyploid animals (and triploid plants) are often asexual (Astaurov 1969; Bierzychudek 1985; Suomalainen 1987; Otto and Whitton 2000). Why polyploidy and asexuality are associated, however, remains unclear (Gregory 2005). To complicate matters, there is growing evidence that ploidy level within presumed obligate asexual lineages is surprisingly variable and complex (Lynch 1984; Belshaw et al. 1999; Castageno-Serrano 2006). Independent origin of asexual lineages from sexuals that themselves vary in karyotype or genome size and occasional fertilization of otherwise asexual individuals are the two most commonly suggested causes for this complexity, especially when ploidy elevation is involved (e.g., amphibians: Bogart and Licht 1986; Christensen and Reyer 2009; arthropods: Sunnucks et al. 1996; Chaplin and Hebert 1997; Normark 1999; fishes: Schultz 1969; Alves et al. 2001; flatworms: D'Souza and Michiels 2009, 2010; plants: Roy 1995; Sharbel and Mitchell-Olds 2001; van Dijk 2003). The link between asexuality and polyploidy and the large body of literature suggesting that the causes and consequences of ploidy level variation can have profound genetic and phenotypic effects (reviewed in Otto and Whitton 2000; Comai 2005; Gerstein and Otto 2009) means that a complete understanding of the distribution and maintenance of sex in many natural systems will often require the characterization of ploidy-level variation and its relationship to mating system.

The most direct and powerful way to study the maintenance of sex is to compare obligately sexual and obligately asexual organisms that coexist in nature and where the asexuals are repeatedly and independently derived from sexual ancestors (Maynard Smith 1978; Neiman and Jokela 2010). This scenario is one where sex is likely to be actively maintained, and permits direct comparisons between sexual and asexual organisms. However, these conditions are very rarely met in natural mixed sexual/asexual systems (Maynard Smith 1978).

The frequent coexistence of sexual and asexual individuals (Winterbourn 1970; Lively 1987, 1992) and evidence for repeated transitions to asexuality (Dybdahl and Lively 1995; Neiman and Lively 2004) are thus why *Potamopyrgus antipodarum*, a New Zealand freshwater snail, is perhaps one of the best natural systems available for studying the maintenance and distribution of sex in nature. Lake populations of *P. antipodarum* in its native range are either entirely or almost entirely female (<10% male; "low male") or ~20-50% male ("high male", Wallace 1992; also see Winterbourn 1970; Lively 1987, 1992; Lively

and Jokela 2002). Allozyme genotyping of females from low- and high-male populations first revealed that the former nearly always reproduced via apomictic parthenogenesis, while the latter were usually sexual (Phillips and Lambert 1989). Soon after, karyotyping of embryos and adults from twelve low male and ten high male populations suggested that mating system covaried with ploidy, but also hinted at ploidy or genome size variation within asexual *P. antipodarum* (Wallace 1992). While *P. antipodarum* from high male lakes were generally diploid (2x = 34), the individuals from low male lakes (including several males) had a chromosome number that ranged between forty-six and fifty-two, and were referred to as "modified triploids." More recently, nucleic acid assays of multiple asexual *P. antipodarum* lineages revealed wide across-lineage variation in per unit mass DNA content within the "triploid" ploidy class (Neiman et al. 2009).

The *P. antipodarum* system thus constitutes a set of natural experiments in the evolution of mating system and ploidy-level variation, and is well-suited for empirical studies aimed at understanding the maintenance and distribution of sex and disentangling the relationships between polyploidy and asexuality. Achieving these goals requires the characterization of within- and among-population variation in ploidy level and an understanding of how ploidy-level variation is connected to variation in mating system. With this in mind, we conducted an extensive survey of ploidy-level variation in New Zealand populations of *P. antipodarum* and evaluated how ploidy level covaried with phylogeographic identity, mating system, and gender.

## Methods

### *Sampling*

We sampled *Potamopyrgus antipodarum* in four North Island and twelve South Island lakes in January 2009 and in six South Island lakes in January 2010 by pushing a kick net through vegetation and washing snails off of rocks (Fig. 1). We also collected samples from each of three depth-structured habitat zones in Lake Alexandrina (Jokela and Lively 1995). There is a well-established gradient of the relative frequency of sexual *P. antipodarum* in this lake, where the shallow and intermediate zones are characterized by mixed sexual/asexual (diploid/triploid), high-male populations, while *P. antipodarum* in the deep zone are usually triploid females (Jokela and Lively 1995; Fox et al. 1996; Table 1). With the exception of the

intermediate and deep samples from Lake Alexandrina, all other samples were taken from shallow regions.

All field-collected snails were transported to the University of Iowa (2009) or the Swiss Federal Institute of Aquatic Science and Technology ("EAWAG") (2010), and housed in aquaria until used for flow cytometry. At this time adult snails (>2.5 mm) were randomly selected from the field collections, sexed (with the presence of a penis used to assign male status), and dissected such that any brooded embryos were removed prior to flow cytometry. We then snap-froze the head in two separate halves (one for flow cytometry and one for DNA extraction). We dissected and froze heads from the first ten males that we identified along with the first 20+ haphazardly selected females from 2009 field collections that had a relatively high relative frequency of males (>20%). For 2009 field collections that had <10% males, we sexed all collected adults (100+ individuals) and dissected and froze heads from all the males (ranging in number from two to seven) for flow cytometry, along with the first 20+ haphazardly selected females. For samples collected in 2010, *P. antipodarum* were sexed and dissected immediately prior to flow cytometry analysis. Since transportation-associated losses of 2010 samples brought to Switzerland were large (~60-90% mortality per sample), all remaining live adults per lake sample were analyzed (fifteen to thirty-eight individuals).

A 2008 pilot study demonstrated that flow cytometry analysis of body (vs. head) tissue of adult diploid male *P. antipodarum* reliably generates a bimodal peak pattern, with fluorescence peaks (in FL1 units) from the DNA-staining dye DAPI centered both at the same location as the diploid head tissue run alone and at about half the FL1 units representing a typical head tissue peak (data not shown; "body tissue peak"). Since the gonads are found in this posterior portion of the *P. antipodarum* body, and since this second peak was never found in association with head tissue run alone, it is likely to represent haploid sperm cells produced by diploid males. We thus also froze the bodies of male *P. antipodarum* sampled in 2009 in order to analyze the nuclear DNA content of the cells contributing to this body tissue peak. A possible signature of ova produced by adult female diploid *P. antipodarum* was not apparent in flow cytometry using body tissue, probably because there are almost certainly orders of magnitude more sperm cells in an adult male than egg cells in an adult female. Bodies of males infected with macroparasites (<10% of all males) were not used for flow cytometry since there was no way to ensure complete removal of the parasite tissue from *P. antipodarum*

body tissue. All *P. antipodarum* tissue was stored at -80°C until DNA extraction and flow cytometry.

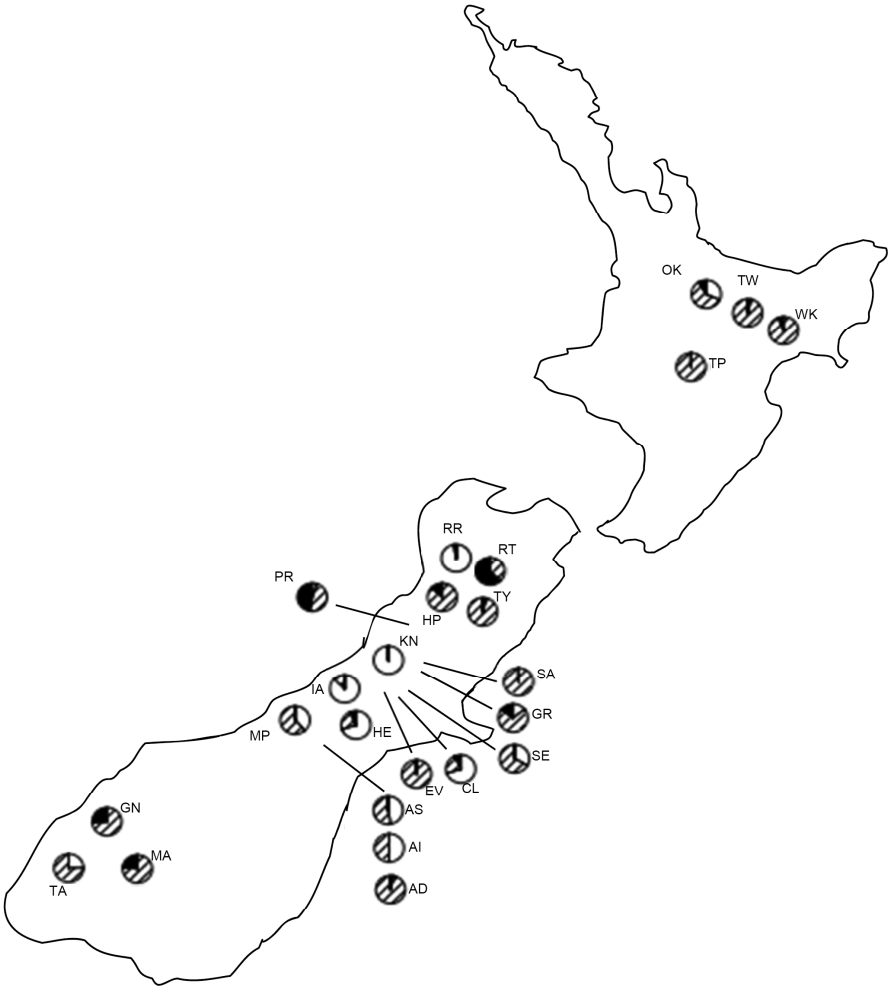


Figure 1. Map of New Zealand showing the locations of and the relative frequency of 2x (white), 3x (stripe), and >3x (black) individuals in the twenty-two lakes (twenty-four samples including the three different depth zones in Lake Alexandrina) from which we collected 860 *P. antipodarum* in 2009 and 2010. Each lake/sample is indicated by the acronym assigned in Table 1. We also used sixteen *P. antipodarum* from laboratory cultures descended from individuals sampled from Lady Lake in New Zealand (2007) and from invasive populations in North America (Lake Superior, July 2007) and Europe (sampled in early 1990's) for mitochondrial cytochrome-b sequencing.

***Flow cytometry***

Samples collected in 2009 were analyzed at the University of Iowa. For these samples, frozen *P. antipodarum* heads were ground in a solution containing 0.2M Tris-HCl (pH 7.5), 4 mM $MgCl_2$, 1% TritonX-100, and 4 μg/mL DAPI. This solution was filtered through a 70 micron nylon sheet, and then run on a Beckman-Coulter Quanta SC MPL flow cytometer. We used the FL1 channel to assess DAPI fluorescence (and thus the DNA content) of *P. antipodarum* cell nuclei under a UV lamp. At the beginning of each flow cytometry run, we calibrated the machine using 20 μL of chicken red blood cells (Lampire Biological Labs, Pipersville, PA) treated and filtered as for *P. antipodarum*. We adjusted the gain so that the chicken standard peak was always centered on 200 FL1 units. Each sample was run until a count of 10,000 events was achieved. We also used flow cytometry on the body tissue of diploid and polyploid males to determine whether polyploid males were similar to diploid males in having a peak of relatively low DNA content nuclei from body tissue that might correspond to sperm. These data were also used to quantify the nuclear DNA content of cells found in body tissue samples.

Snails collected in 2010 were analyzed at EAWAG. We ground fresh *P. antipodarum* heads in 100 μL of CyStain UV Ploidy solution (Partec, Muenster, Germany). We then added an additional 700 μL of CyStain UV Ploidy solution to the ground snail tissue, and filtered the solution through 30 micron Partec CellTrics mesh. Nuclear DNA content was measured in the FL4 channel of a Partec Ploidy Analyzer PA-II equipped with an HBO mercury arc lamp. As a size standard we used *Drosophila melanogaster* (OR-strain) male heads, which were treated and filtered the same way as *P. antipodarum* samples. Four or five of these *Drosophila* extracts were then mixed together and 100 to 200 μL of this solution was added to each snail sample prior to flow cytometry measurement. Each sample was run until a count of 17,000 events was achieved. We tested the repeatability of the flow cytometry procedure by measuring the fluorescence of separately prepared head halves from the same individual *P. antipodarum* (2009 samples) and by measuring the fluorescence of individual *P. antipodarum* samples twice during the course of a single flow cytometry run (2010 samples). The two measures were significantly and positively correlated for both 2009 (N = eight individuals; Pearson's correlation coefficient = 0.998, $p < 0.0001$) and 2010 (N = twenty-five individuals; Pearson's correlation coefficient = 0.999, $p < 0.0001$). Since the repeatability analysis from 2010 included individuals from multiple ploidy levels, we also conducted these analyses

separately within the 2x (N = five) and 3x (N = eighteen) individuals, with very similar results (2x: Pearson's correlation coefficient = 0.991, $p = 0.001$; 3x: Pearson's correlation coefficient = 0.998, $p < 0.001$). There were only two >3x individuals in the 2010 repeatability dataset, meaning that we did not have a large enough sample size to perform a meaningful assessment of whether the two flow cytometry measurements from each individual were correlated.

*Data Analysis*

In order to avoid including cell debris and clumped cells in data analyses, we first used the Beckman Quanta Analysis software (2009 samples) and Partec FloMax software (2010 samples) to confine data analysis for each flow cytometry sample to the peak of data points corresponding to intact nuclei of single cells in growth phase 1. We did the same for the chicken and *Drosophila* standards and for the male body tissue peaks. We then calculated the mean fluorescence (in FL1 units for 2009 samples, in FL4 units for 2010 samples) for this peak region for each sample. For comparison between different runs on the same machine, we standardized each data point by dividing the mean FL1 (FL4) value for each sample by the mean fluorescence of the peak of chicken red blood cell (or *Drosophila*, for 2010 samples) standard used to calibrate that particular run. The *Drosophila* standard was mixed with each sample, meaning that for samples run in 2010, we were able to calibrate each sample with a simultaneously run standard.

In order to combine the 2009 and 2010 datasets, we divided each sample's mean standardized fluorescence value by the mean fluorescence value of a haploid genome in its dataset. We estimated the mean haploid genome fluorescence value by using the frequency distributions of the two datasets, which each showed two distinct peaks of fluorescence values (and thus nuclear DNA content) corresponding to putatively diploid and triploid *P. antipodarum*, to delineate diploid classes of individuals. We calculated the mean fluorescence value of the diploid class, and divided this value by two to generate an estimate of mean haploid genome fluorescence for each dataset. The 2009 dataset had a broader distribution of fluorescence values than the 2010 dataset, probably because the 2009 dataset involved a larger and different set of lakes (including Lake Rotoiti, from which 35 of the 77 individuals classified as >3x and 58% of the individuals representing the top 0.5% of the distribution of nuclear DNA contents were sampled), and because 2010 samples were run fresh and mixed with a standard. Therefore, we used a well-defined local minimum between 0.96-0.99 FL1

units in the 2009 dataset to designate individuals with FL1 values ≤ 0.96 as "2x." For the 2010 dataset, which was distinctly trimodal, we classified the 45 individuals with the lowest FL1 values (lowest FL4 values) as 2x (mean FL4 = 3.994 units +/- 0.114 SD). We divided each of these mean fluorescence values for the 2x classes by two in order to generate a 1x fluorescence value for each dataset. The standardized fluorescence value for each sample was then divided by its 1x fluorescence value (2009: 0.402; 2010: 1.995).

After assigning individuals to ploidy levels (see below), we used a one-way random effects ANOVA (as implemented within PASW 17; SPSS Inc. 2009) to determine whether mean fluorescence (i.e., nuclear DNA content) within each ploidy level differed by population (either lake, or habitat within a lake). Ploidy classes containing fewer than three individuals for a given population were excluded from these analyses. As most populations did not have all ploidy levels, we fitted random effects ANOVA models for each ploidy level. We also conducted variance component estimation using the MINQUE method to estimate within- and among- population variance components.

Similar analyses were conducted for body tissue samples from diploid and triploid males. Since there were only two >3x males for which we collected body tissue data, we did not include these two males in these analyses. We analyzed among-population differences in the DNA content of the cells represented by this body tissue peak for each ploidy level using individual as a random factor nested in population of origin, which was also treated as a random factor.

Comparisons of the mean and variance of the nuclear DNA content of body tissue cells in triploid and diploid males and of the mean nuclear DNA content in head vs body tissue in triploid males could also provide preliminary information about gametogenesis in polyploid males, provided that future studies verify that this low-fluorescence peak generated by male body tissue is indeed from sperm cells. We compared the means of the nuclear DNA content of body tissue in diploid vs triploid males by fitting a model where ploidy of the male (diploid, triploid) was treated as a fixed factor. Since we did not find triploid males in all of our lake samples, we could not estimate the interaction between ploidy and population of origin. We compared the means of the nuclear DNA content of body vs head tissue in triploid males with a paired-values t-test (as implemented in PASW 17), where the mean FL1 values of head and body tissue were paired within their source individual. We compared the

variances of the nuclear DNA content in the body tissue of diploid and triploid males by using a Levene's test of equality of variances.

***DNA extraction, PCR, and sequencing***

Total DNA was isolated with the Qiagen DNeasy Plant kit from each individual collected and used for flow cytometry in 2009. We also extracted DNA from sixteen individual *P. antipodarum* from fifteen lab cultures housed at the University of Iowa that are descended from individuals collected from various New Zealand lakes between 2003-2008 and from invasive North American (Lake Superior, collected July 2007) and European (collected in the early 1990s) populations. Flow cytometry had been previously used to establish ploidy of the individual cultures, and was used to verify the ploidy of the individuals used for DNA extraction by the University of Iowa 2009 methods described above for head tissue.

A portion of the mitochondrial cytochrome-b gene was amplified from the extracted DNA using primer pair A (forward-CTTGAATGACAGCAAGAAGT; reverse-GTTGCTAGGCTGCTAAGTGA) or B (forward-GTTGACTTACCWGCWCC; reverse-AGGGCATGCCCCDATTCA). Primer pair A amplified a 1.7 kb fragment in a reaction containing 1 U Phusion Polymerase (New England BioLabs, Ipswich, MA, USA), 1x HF Buffer, 0.2 mM each dNTP, 0.5 μM each primer, and 10-20 ng genomic DNA with cycling parameters of 1 cycle of 98°C for 2 minutes, 35 cycles of 98°C for 20 seconds, 62°C for 30 seconds, 72°C for 1 minute, and 1 cycle of 72°C for 10 minutes. Primer pair B amplified a 970 bp fragment in a reaction containing 2 U Biolase Polymerase (Bioline USA Inc., Taunton MA, USA), 1X $NH_4$ Buffer, 2.0 mM $MgCl_2$, 0.4 mM each dNTP, 1.0 μM each primer, and 10-20 ng genomic DNA with cycling parameters of 1 cycle of 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute, and 1 cycle of 72°C for 10 minutes. PCR products were purified using QIAquick PCR Purification (Qiagen Inc., Valencia, CA, USA). Fragments were sequenced from one or more primers from Primer Pair A, Primer Pair B, Primer C (AATTTGTCWATYTGATGA), or Primer D (AAAATATCATTCNGGYTG) using BigDye version 3.1 (Applied Biosystems, Foster City, CA, USA) and an ABI 3730 (Applied Biosystems).

*Data analysis*

Cytochrome-b sequences were edited and aligned in BioEdit (Hall 1999). We trimmed the sequences to the 718 bp that we were able to reliably sequence in most individuals. We then used the "Findmodel" application of Modeltest 3.7 (Posada and Crandall 1998) to select the model of nucleotide substitution that best fitted the 513 individual, 718 bp dataset. Finally, we used MEGA 4.0 (Tamura et al. 2007) to run 1000 replicates of a neighbor-joining bootstrap procedure under the Modeltest-specified model, Tamura-Nei, to evaluate the statistical support of the cytochrome-b genealogy topology.

## Results

### *Flow cytometry*

We quantified genome size in 860 adult *P. antipodarum* from twenty-four field samples (N = 14-64). Samples from 2009 (670 *P. antipodarum* individuals; 510 females, 160 males) were analyzed at the University of Iowa, and samples from 2010 (190 *P. antipodarum* individuals; 165 females, 25 males) were analyzed at EAWAG. We also estimated the DNA content of nuclei in the low-fluorescence FL1 peak generated from body tissue content in sixty-six males (forty-five diploid, nineteen triploid, two >3x) collected in New Zealand in 2009 at the University of Iowa.

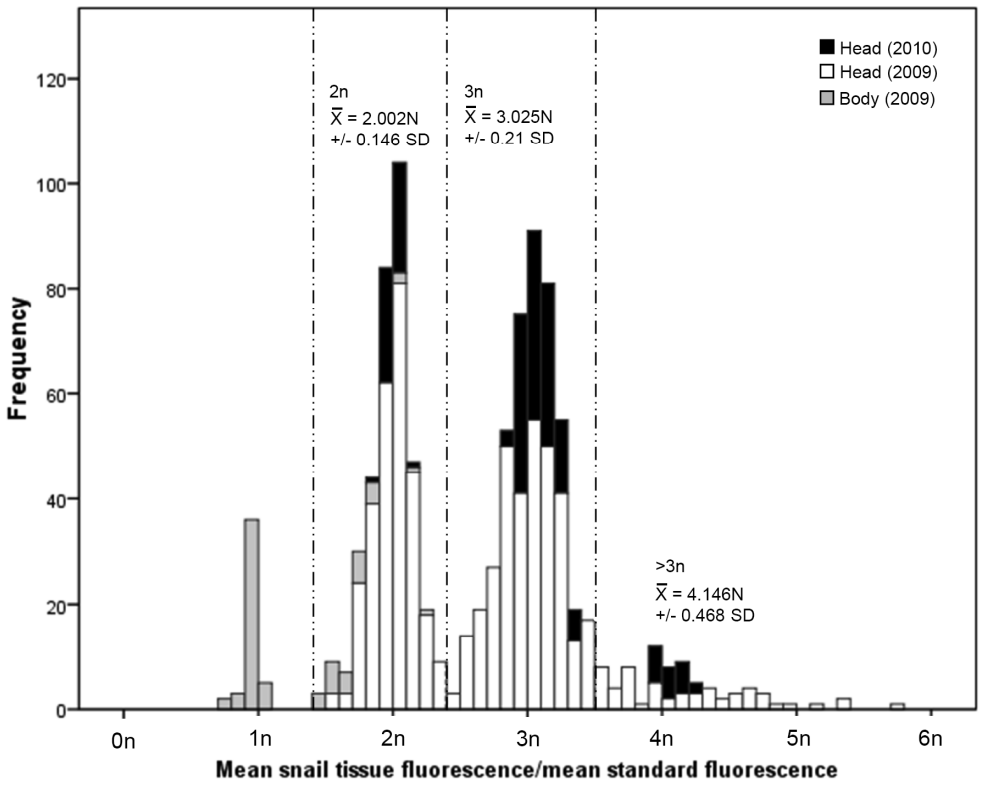


Figure 2. Histogram showing the distribution of mean fluorescence of the peak corresponding to DAPI-labeled intact nuclei from single cells in growth phase 1 generated from the head tissue of 860 individual *P. antipodarum* and from body tissue of 66 male *P. antipodarum*. Each fluorescence value from each individual was standardized by dividing by the mean fluorescence of the chicken red blood cell standard (2009) or the male *Drosophila* head standard (2010) used for that particular run. We then used the distribution of fluorescence values from each of the two datasets (2009 and 2010) to designate 2x ploidy classes for each dataset, and then generated a fluorescence value corresponding to mean haploid nuclear DNA content for each dataset by dividing the mean fluorescence value of the individuals in the 2x ploidy class by two. We then divided the fluorescence values of each individual in each dataset by the mean haploid nuclear DNA content value from its dataset to produce a single pooled dataset where each individual's fluorescence value was scaled by the mean haploid nuclear DNA content for its dataset. The vertical dashed lines indicate the points of demarcation between the 2x and 3x designation (</= 2.39x) and the 3x and >3x designation (</= 3.5x). We classified 38.26% (329) individuals as 2x (mean nuclear genome content = 2.002x +/- 0.146 SD), 52.79% (454) individuals as 3x (mean = 3.025n +/- 0.21 SD), and 8.95% (77) individuals as >3x (mean = 4.146x +/- 0.468 SD). Data from body tissue were not included in the calculation of these means.

The pooled frequency distribution of the 2009 and 2010 head tissue datasets showed two distinct peaks of fluorescence values (and thus nuclear DNA content) corresponding to putatively diploid and triploid *P. antipodarum*, as expected from previous cytological (Wallace 1992) and population genetic (Fox et al. 1996) data. Surprisingly, the dataset also contained individuals with nuclear DNA content clearly exceeding 3x (Fig. 2). We used local minima at 2.39N and 3.5N to designate individuals with nuclear genome content ≤ 2.39N as "2x," individuals with nuclear genome content >2.39N but ≤ 3.5N as "3x," and individuals with nuclear genome content > 3.5 as ">3x." Fifty males were classified as >2x, and are referred to as "polyploid males." All of the males sampled from low-male populations (for details see Table 1; also see Wallace 1992; Lively and Jokela 2002) were polyploid, with the exception of four diploid males from a Lake Grasmere sample that was dominated by triploid females. As shown in Table 1, we found >3x individuals in ten lakes, six of which had no diploid males. One South Island lake sample, Rotoiti, was made up of predominantly >3x individuals (79.5% of the 44 snails sampled) (Table 1). Three other South Island lake samples had ~25% >3x individuals (Lake Gunn, South Mavora, and Lake Poerua) (Table 1). Nearly all >3x individuals were female, with only three of the seventy-seven >3x individuals found to be male (3.9%). This is even a lower relative frequency of males than for triploid asexual *P. antipodarum* (10.4%; 47 of 454), indicating that >3x individuals are likely to also be asexual.

There was significant among-population variance in mean nuclear DNA content within each ploidy class (Table 2, Fig. 3), varying from 32% in diploids to 23% in triploids and 11% in >3x individuals. Such variation is likely to be linked to variation in karyotype and/or genome size (e.g., Burton and Husband 1999; Normark 1999; Sharbel and Mitchell-Olds 2001; Šmarda and Bureš 2006), though we cannot exclude other potential sources of variation in DAPI staining intensity (reviewed in Leitch and Bennett 2004**;** e.g., variation in nucleotide composition and/or satellite DNA content, Bosco et al. 2007). Among-population variance in body tissue DNA content was even higher (82%), which we discuss in detail below.

Finally, we used Pearson's correlation to determine whether the within-population variance in genome size between diploids and triploids was associated. We found that populations with large variance in diploid genome sizes also had large variance in triploid genome sizes ($r = 0.83$, N = 10, $p = 0.003$). Estimates of within-population correlations between diploid and >3x genome size and variances were not possible because of the low

number of lakes containing enough diploids and >3x individuals to perform meaningful analyses.

Table 1. Characteristics of the New Zealand lake populations and laboratory cultures used for flow cytometry and sequencing

| Source (acronym) | Type | N (flow) | ♂ # | ♀ # | #2x/#3x/#>3x | % >3x | #2x♂/#3x♂/#>3x♂ | % ♂ 2x (all ♂) | % polyploid ♀ (all ♀) | % triploid ♀ (all ♂) | % ♂ >3x (all ♂) | #2x♂/3x♂/>3x♀ | % ♀2x (all ♀) | % polyploid ♂ (all ♂) | % ♀3x (all ♀) | % >♀3x (all ♂) | N (sequence) | Haplotypes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Field-Collected (2009)*** [a] | | | | | | | | | | | | | | | | | | |
| Alexandrina Isoetes (AI) | High male | 60 | 36 | 24 | 42/18/0 | 0.00 | 18/18/0 | 50.00 | 50.00 | 50.00 | 0.00 | 24/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 41 | 01A, 10A, 25A, 30A, 30B, 30E |
| Alexandrina Deep (AD) | Low male | 54 | 48 | 6 | 2/52/0 | 0.00 | 2/46/0 | 4.17 | 95.83 | 95.83 | 0.00 | 0/6/0 | 0.00 | 100.00 | 100.00 | 0.00 | 54 | 01A , 10A, 30A, 50 |
| Alexandrina Shallow (AS) | High male | 50 | 40 | 10 | 29/21/0 | 0.00 | 19/21/0 | 47.50 | 52.50 | 52.50 | 0.00 | 10/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 40 | 01A , 10A, 25A, 30A, 30C, 30E |
| Clearwater (CW) | High male | 64 | 30 | 34 | 54/8/2 | 3.13 | 21/7/2 | 70.00 | 30.00 | 23.33 | 6.67 | 33/1/0 | 97.06 | 2.94 | 2.94 | 0.00 | 26 | 01A, 30A |
| Evelyn (EV) | Low male | 25 | 20 | 5 | 0/25/0 | 0.00 | 0/20/0 | 0.00 | 100.00 | 100.00 | 0.00 | 0/5/0 | 0.00 | 100.00 | 100.00 | 0.00 | 20 | 01A, 49 |
| Grasmere (GR) | Low male | 28 | 21 | 7 | 7/19/3 | 10.71 | 3/15/3 | 14.29 | 85.71 | 71.43 | 14.29 | 4/3/0 | 57.15 | 42.85 | 42.85 | 0.00 | 27 | 01A, 30A |
| Heron (HE) | High male | 51 | 30 | 21 | 41/8/2 | 3.92 | 21/7/2 | 70.00 | 30.00 | 23.33 | 6.67 | 20/1/0 | 95.24 | 4.76 | 4.76 | 0.00 | 36 | 01A, 30A, 52 |
| Kaniere (KN) | High male | 30 | 20 | 10 | 30/0/0 | 0.00 | 20/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 10/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 25 | 27A, 30D, 53, 54, 55, 56 |
| Okareka [c] (OK) | Low male | 34 | 34 | 0 | 11/20/3 | 8.57 | 11/20/3 | 32.35 | 67.65 | 58.82 | 8.82 | 0/0/0 | NA | NA | NA | NA | 14 | 01A, 08A, 37A, 37C, 37E, 64, 68 |
| Poerua [c] (PR) | Low male | 21 | 19 | 2 | 1/11/9 | 42.86 | 1/9/9 | 5.26 | 94.74 | 47.37 | 47.37 | 0/2/0 | 0.00 | 100.00 | 100.00 | 0.00 | 19 | 01A |
| Rotoiti (RT) | Low male | 44 | 42 | 2 | 0/9/35 | 63.54 | 0/16/26 | 0.00 | 100.00 | 38.10 | 61.90 | 0/0/2 | 0.00 | 100.00 | 0.00 | 100.00 | 18 | 01A, 37B, 48 |
| Rotorua (RR) | High male | 46 | 36 | 10 | 45/1/0 | 0.00 | 35/1/0 | 97.22 | 2.78 | 2.78 | 0.00 | 10/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 39 | 01A, 09A, 09B, 09C |
| Sarah (SA) | Low male | 26 | 20 | 6 | 0/26/0 | 0.00 | 0/20/0 | 0.00 | 100.00 | 100.00 | 0.00 | 0/6/0 | 0.00 | 100.00 | 100.00 | 0.00 | 21 | 01A, 37A |
| Selfe (SF) | High male | 40 | 30 | 10 | 20/20/0 | 0.00 | 10/20/0 | 33.33 | 66.67 | 66.67 | 0.00 | 10/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | 33 | 01A, 9B, 27A, 27B |
| Tarawera [c] (TW) | Low male | 28 | 24 | 4 | 1/27/0 | 0.00 | 1/23/0 | 4.17 | 95.83 | 95.83 | 0.00 | 0/4/0 | 0.00 | 100.00 | 100.00 | 0.00 | 21 | 01A, 37A, 61, 62, 63, 66 |
| Taupo (TP) | Low male | 23 | 20 | 3 | 0/23/0 | 0.00 | 0/20/0 | 0.00 | 100.00 | 100.00 | 0.00 | 0/3/0 | 0.00 | 100.00 | 100.00 | 0.00 | 16 | 37A, 37E, 64, 65, 66 |
| Taylor [c] (TY) | Low male | 23 | 19 | 4 | 1/22/0 | 0.00 | 1/18/0 | 5.26 | 94.74 | 94.74 | 0.00 | 0/4/0 | 0.00 | 100.00 | 100.00 | 0.00 | 22 | 01A |
| Waikaremoana (WK) | Low male | 23 | 18 | 5 | 0/21/2 | 8.69 | 0/17/1 | 0.00 | 100.00 | 94.44 | 5.54 | 0/4/1 | 0.00 | 100.00 | 80.00 | 20.00 | 25 | 08A, 22A, 37A, 57, 58, 59, 60 |
| ***Field-Collected (2010)*** | | | | | | | | | | | | | | | | | | |
| Gunn (GN) | Low male | 35 | 35 | 0 | 0/26/9 | 25.71 | 0/26/9 | 0.00 | 100.00 | 74.29 | 25.71 | 0/0/0 | NA | NA | NA | NA | NA | NA |
| Haupiri (HP) | High male | 38 | 30 | 8 | 3/31/4 | 10.53 | 0/26/4 | 0.00 | 100.00 | 86.67 | 13.33 | 3/5/0 | 37.50 | 62.50 | 62.50 | 0.00 | NA | NA |
| Ianthe (IA) | High male | 29 | 16 | 13 | 27/2/0 | 0.00 | 14/2/0 | 87.50 | 12.50 | 12.50 | 0.00 | 13/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | NA | NA |
| Mapourika (MP) | High male | 14 | 13 | 1 | 6/8/0 | 0.00 | 5/8/0 | 38.46 | 61.54 | 61.54 | 0.00 | 1/0/0 | 100.00 | 0.00 | 0.00 | 0.00 | NA | NA |
| South Mavora (MA) | Low male | 35 | 35 | 0 | 0/27/8 | 22.86 | 0/27/8 | 0.00 | 100.00 | 77.14 | 22.86 | 0/0/0 | NA | NA | NA | NA | NA | NA |
| Te Anau [c] (TA) | Low male | 39 | 36 | 3 | 9/30/0 | 0.00 | 9/27/0 | 25.00 | 75.00 | 75.00 | 0.00 | 0/3/0 | 0.00 | 100.00 | 100.00 | 0.00 | NA | NA |
| ***Laboratory Cultures (ploidy)*** [b] | | | | | | | | | | | | | | | | | | |
| Ianthe 10 (IN10; 2x) | Sex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 41A |
| Ianthe 41 (IN41; 2x) | Sex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 41B |
| Lady 21 (LD21; 2x) | Sex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 30A |
| Lady 24 (LD24; 2x) | Sex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 30A |
| Lady 51 (LD51; 2x) | Sex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 51 |
| Alex Isoetes 1 (AI1; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 01A |
| Alex Isoetes 67 D (AI67D; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 01A |
| Alex Isoetes 7 (AI7; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 22A |
| Alex Isoetes B52 (AIB52; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 01A |
| Denmark A (DKA; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 22A |
| Gunn (GN; >3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 20A |
| Mapourika 75 (MP75; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 37A |
| Superior (SP; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 22A |
| Waikaremoana 90 $s^{4}$ (WK90; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 2 | 36A, 67 |
| Wales C (WLC; 3x) | Asex | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 1 | 37D |

Table 1 footnotes:

[a]For the 2009 field collections, all males found in a sample of 200+ snails were used for flow cytometry in low male lakes, while at least 10 males were used in high male lakes. Male relative frequency was defined in field collections by the relative frequency of males relative to all adults within a random sample of at least 100 adults taken from the lake collections (low = <10% males/sample; high = >20% males/sample; Lively and Jokela 2002).

[b]Ploidy was previously established for laboratory cultures and was verified with flow cytometry of the individual(s) used for DNA extraction.

[c]There were no diploid males found in these lakes in samples of 200+ snails, indicating that females classified as diploids in these lakes may be asexual.

[d]Ancestors of this culture were collected in 2003, and the culture was founded with multiple individuals.

[e]Ancestors of this culture were collected in the early 1990s, and the culture was founded with multiple individuals.

*Body tissue nuclear DNA content*

The mean DNA content of the nuclei represented by the low-fluorescence FL1 peak generated from triploid male body tissue was significantly lower than the mean nuclear DNA content estimated from their head tissue ($t = 21.604$, $df = 18$, $p < 0.0001$), suggesting that triploid males might be producing reduced sperm. Even so, the nuclear DNA content in the body tissue peak from triploid males was significantly higher than that from diploid males (Fig. 4, $F_{2, 61} = 251.951$, $p < 0.0001$), suggesting that sperm produced by triploid males usually has ploidy levels higher than haploidy (also see Guo and Allen 1994 Biol. Bulletin). This peak also had ~550% higher standard deviation in triploid vs diploid males (0.242 SD vs 0.044 SD; Levene's test for equality of variances, $F_{2, 62} = 27.722$, $p < 0.0001$), pointing to the possibility of wide variation in nuclear DNA content in sperm produced by triploids.

While there was significant among-population variation in nuclear DNA content in body tissue for diploid males (2.8% of total variance; $F = 3.37$, $p = 0.018$), the variation amongst individuals was approximately four times greater (8.7% of total variance; $F = 451.00$, $p < 0.001$). The situation was similar for triploid males, where individual-level variation again contributed much more to total variance (22.6% of total variance; $F = 1470.00$, $p < 0.001$) than population-level variation (1.1% of total variance; $F = 1.05$, $p = 0.436$) for triploid males (Fig. 4). The relatively high contribution of individual-level variance to total variance in body tissue nuclear DNA content in combination with wide among-

population variation in the relative frequency of different ploidy levels thus can explain the variance in body tissue DNA content among populations reported above.

Table 2. Analysis of within and among population variance in individual DNA content by ploidy. "Body" includes data from body tissue from males at different ploidy levels.

| model* | MS (population / error) | DF (population / error) | *F* | *p* | Var (%)** |
|---|---|---|---|---|---|
| Body | 0.493 / 0.019 | 8 / 54 | 26.54 | <0.001 | 82.0 |
| 2x | 0.036 / 0.004 | 13 / 312 | 9.49 | <0.001 | 31.8 |
| 3x | 0.027 / 0.004 | 21 / 431 | 6.84 | <0.001 | 23.9 |
| >3x*** | 0.032 / 0.009 | 9 / 67 | 3.63 | 0.001 | 11.1 |

* Models were fitted as a random effects model where variance in DNA content was divided into within and among population components. Ln-transformed data were used to achieve homoscedastic variances among populations.

**Among population variance components are estimated using untransformed data by applying the MINQUE method available in PASW 17. This method is suitable for heteroscedastic variance and yields unbiased variance estimates for unbalanced data (Sahai and Ojeda 2005).

*** Homogeneity of variance assumption violated despite the ln-transformation, so the test should be interpreted with caution.

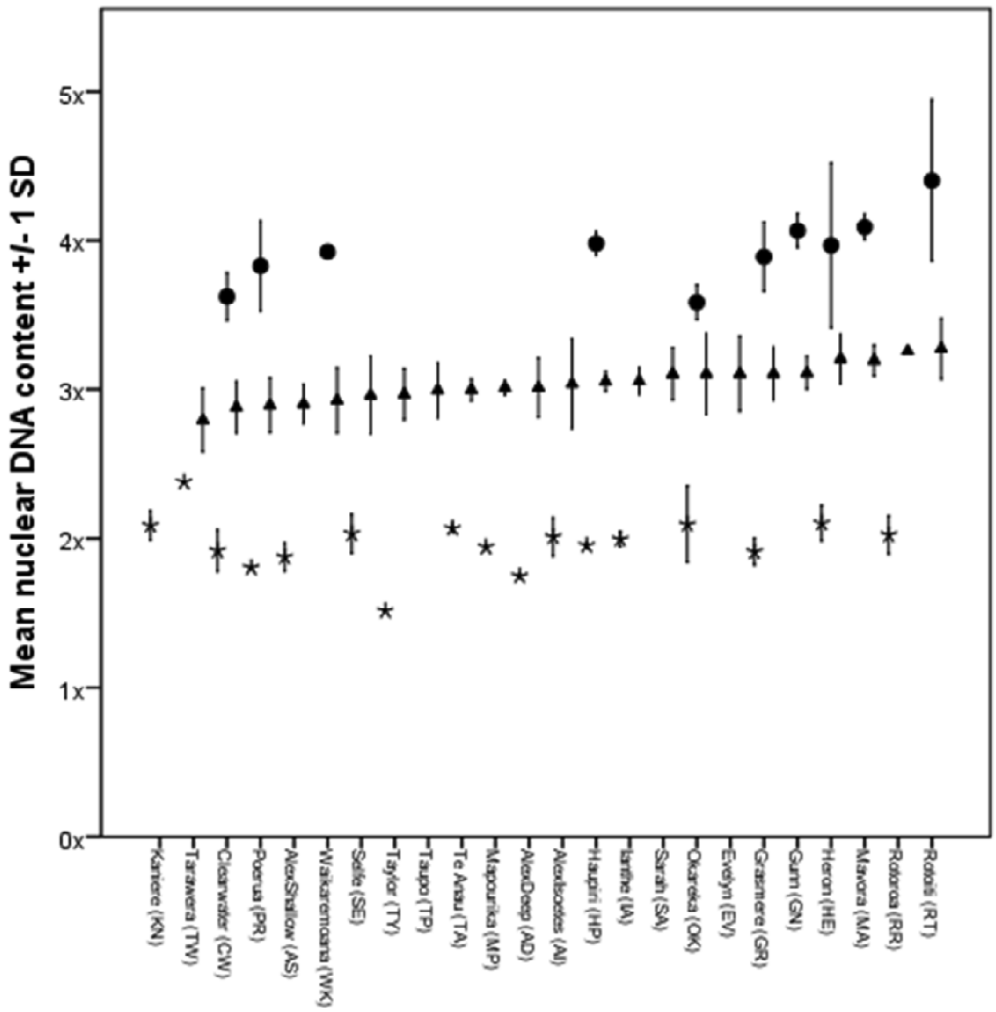


Figure 3. Across lake/habitat variation in nuclear DNA content within and across ploidy levels (2x = star, 3x = open triangle, >3x = filled circle). Lakes/habitats are rank-ordered by increasing mean 3x nuclear DNA content; there were no 3x individuals sampled from Kaniere. Ploidy levels represented by a single individual within a given lake sample (2x individuals from Tarawera, Poerua, and Taylor, and the 3x individual from Rotoroa) were not included within statistical analyses requiring replication within ploidy levels within lakes. There was both a significant effect of lake/habitat on nuclear DNA content values within all three ploidy levels and clear demarcation between the three ploidy levels when lake/habitat was taken into account.

***Cytochrome-b haplotypes***

We sequenced 718 bp of mitochondrial cytochrome-b in 513 individuals for which we also had measured nuclear DNA content (summarized in Table 3). We detected eighty-one polymorphic sites, and identified forty-five haplotypes (Genbank accession #s JF518834-JF518877) within four major clades that were separated from one another by > 1.5% sequence divergence (Fig. 5). Most of the haplotypes were rare (< 1 % of individuals). Twelve haplotypes were represented by more than five individuals ("common"; >1% of individuals). Five of these haplotypes were completely confined to diploid individuals and additional three were dominated by diploids (>88% diploids). Three of the common haplotypes were confined to triploid and >3x individuals and one (1A) was dominated by triploids and >3x individuals (98%). Haplotype 1A was the most common haplotype in the dataset, found in 46% of individuals. This haplotype was significantly more common in triploids, found in 77.1% of triploid individuals vs 3.7% of diploids (Fisher's exact test, $p < 0.0001$).

We generated cytochrome-b haplotypes for twenty-five polyploid males from nine lakes, and found five different mtDNA haplotypes. These haplotypes were represented in three of the four major clades, suggesting that polyploid male *P. antipodarum* are of multiple independent phylogenetic origins. Seventeen (68%) of these males had the triploid-dominated haplotype 1A, while the remainder of the males had haplotypes from two well-supported clades (B and C) in which we did not detect diploid males (also see Neiman et al. 2005). The thirty-one individuals with >3x ploidy that we characterized at cytochrome b were represented by six haplotypes (including three of the five where we detected polyploid males) across three clades, suggesting that ploidy levels above triploidy have arisen multiple times in *P. antipodarum*. Twenty-six (83.9%) of these individuals had haplotype 1A and two others had haplotypes within clade A, while the remaining three >3x individuals had haplotypes from clades B (one individual) and D (two individuals).

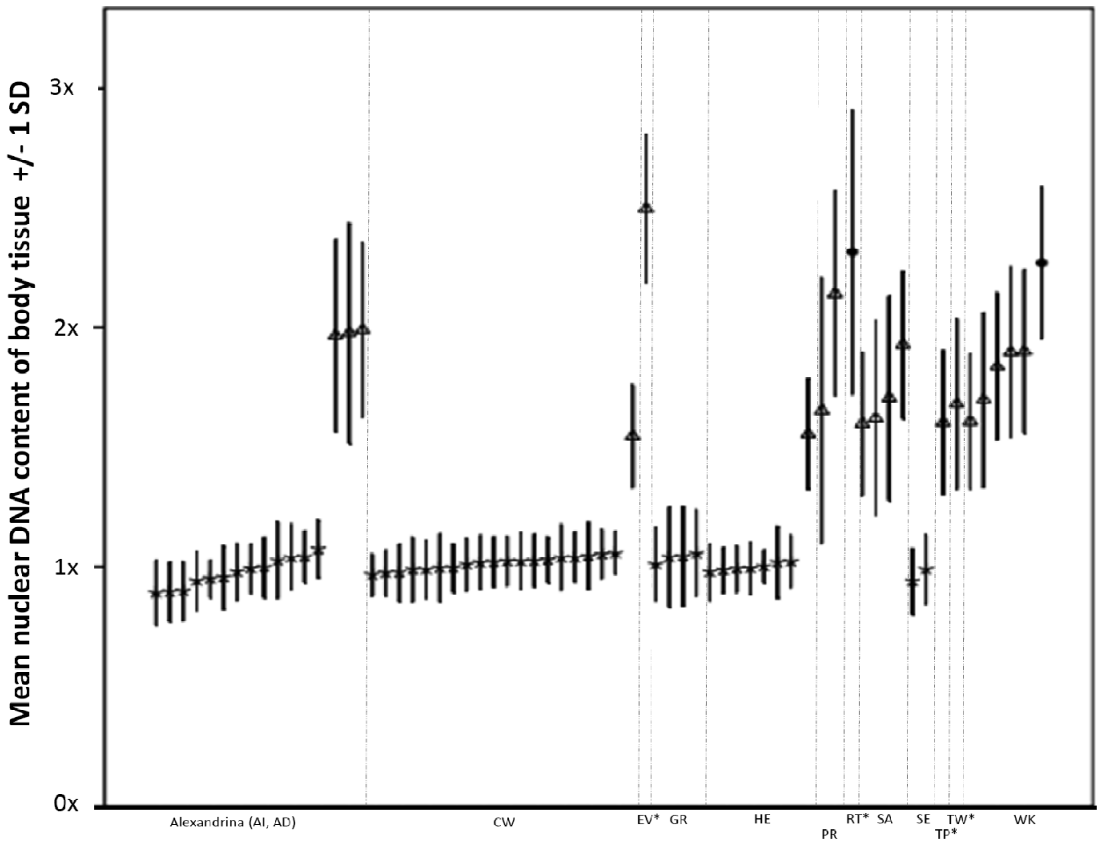


Figure 4. The distribution of the nuclear DNA content of the low FL1 peak from body tissue (standardized with chicken red blood cells) from forty-five 2x males (star), nineteen 3x males (open triangles), and two >3x males (filled circles) sampled from twelve New Zealand lakes. Male somatic ploidy was previously determined using head tissue. Datapoints representing each individual are rank-ordered within lake and ploidy level from smallest to largest. Each lake is indicated by the acronym assigned in Table 1. Individual lake acronyms followed by "*" were not used for statistical analyses requiring replication within lakes since we only generated body tissue data from one individual from each of these lake samples.

Table 3. Characteristics of the 45 different haplotypes identified in the 513 individual *P. antipodarum* sequenced at 718 bp of mitochondrial cytochrome-b.

| Haplotype[a] | N | % Polyploid (# Polyploid) | % Polyploid ♀ ( # ♀) | % Polyploid ♂ (#♂) | %>3x (# >3x) | Lake and/or Culture |
|---|---|---|---|---|---|---|
| 01A | 235 | 97. 44 (228) | 98.13 (224) | 95.00 (20) | 10.68 (25) | >10[c] |
| 08A | 2 | 100.00 (2) | NA (0) | 100.00 (2) | 50.00 (1) | OK, WK |
| 09A | 29 | 0.00 | 0.00 (23) | 0.00 (6) | 0.00 (0) | RR |
| 09B | 11 | 0.00 | 0.00 (5) | 0.00 (6) | 0.00 (0) | RR, SF |
| 09C | 2 | 0.00 | 0.00 (2) | NA (0) | 0.00 (0) | RR |
| 10A | 17 | 5.88 (1) | 8.33 (12) | 0.00 (5) | 0.00 (0) | AD, AI, AS |
| 20A | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 100.00 (1) | GN |
| 22A | 13 | 100.00 (13) | 100.00 (11) | 100.00 (2) | 7.7 (1) | AI7, DKA, SP, WK |
| 25A | 9 | 11.11 (1) | 16.67 (6) | 0.00 (3) | 0.00 (0) | AI, AS |
| 27A | 12 | 8.33 (1) | 14.29 (7) | 0.00 (5) | 0.00 (0) | KN, SF |
| 27B | 2 | 0.00 | 0.00 (2) | NA (0) | 0.00 (0) | SF |
| 30A | 64 | 0.00 | 0.00 (43) | 0.00 (21) | 0.00 (0) | AD, AI, AS, CW, GR, HE, LD21, LD24 |
| 30B | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | AI |
| 30C | 3 | 0.00 | 0.00 (3) | NA (0) | 0.00 (0) | AS |
| 30D | 20 | 0.00 | 0.00 (13) | 0.00 (7) | 0.00 (0) | KN |
| 30E | 6 | 0.00 | 0.00 (4) | 0.00 (2) | 0.00 (0) | AI, AS |
| 36A | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | WK90 |
| 37A | 32 | 100.00 (29[b]) | 100.00 (30) | 100.00 (2) | 0.00 (0) | MP75, OK, SA, TW, TP, WK |
| 37B | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 100.00 (1) | RT |
| 37C | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | OK |
| 37D | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | WLC |
| 37E | 3 | 100.00 (3) | 100.00 (3) | NA (0) | 0.00 (0) | OK, TP |
| 41A | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | IN10 |
| 41B | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | IN41 |
| 48 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 100.00 (1) | RT |
| 49 | 2 | 100.00 (2) | 100.00 (2) | NA (0) | 0.00 (0) | EV |
| 50 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | AD |
| 51 | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | LD51 |
| 52 | 3 | 0.00 | 0.00 (2) | 0.00 (1) | 0.00 (0) | HE |
| 53 | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | KN |
| 54 | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | KN |
| 55 | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | KN |
| 56 | 1 | 0.00 | 0.00 (1) | NA (0) | 0.00 (0) | KN |
| 57 | 3 | 100.00 (3) | 100.00 (3) | NA (0) | 0.00 (0) | WK |
| 58 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | WK |
| 59 | 2 | 100.00 (2) | 100.00 (2) | NA (0) | 0.00 (0) | WK |
| 60 | 4 | 100.00 (4) | 100.00 (2) | 100.00 (2) | 0.00 (0) | WK |
| 61 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | TW |
| 62 | 2 | 100.00 (2) | 100.00 (2) | NA (0) | 0.00 (0) | TW |
| 63 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | TW |
| 64 | 10 | 100.00 (7[b]) | 100.00 (10) | NA (0) | 0.00 (0) | OK, TP |
| 65 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | TP |
| 66 | 5 | 100.00 (5) | 100.00 (5) | NA (0) | 0.00 (0) | TW, TP |
| 67 | 1 | 100.00 (1) | 100.00 (1) | NA (0) | 0.00 (0) | WK90 |
| 68 | 2 | 100.00 (1[b]) | 100.00 (2) | NA (0) | 0.00 (0) | OK |

[a]Haplotype numbers ending in a letter contain 431 bp that are a perfect match for the haplotype with the same number reported in Neiman and Lively (2004). Haplotypes that share a number but end in a different letter vary at a site(s) in the additional 287 bp sequenced here. Haplotypes that do not end in a letter did not contain a 431 bp segment that was a perfect match for any of the haplotypes reported in Neiman and Lively (2004), and were assigned a new number.

[b]No diploid males found in source lakes or with these haplotypes, so these haplotypes were treated as "triploid" (i.e., asexual) in Fig. 5.

[c]This is the "common" haplotype reported in Neiman and Lively (2004), and was found in all lakes sampled in 2009 except KN, TP, and WK. This common haplotype is also represented by the AI1, AIB52, and AI67D cultures.

**Discussion**

We documented the geographically widespread occurrence and multiple phylogenetic origins of lineages containing polyploid males and/or >3x *P. antipodarum*. This evidence for repeated evolutionary transitions to both types of individuals suggests that they do not represent distinct taxa, but are instead generated by many independent events within *P. antipodarum*. The relative frequency of >3x individuals, which were found in nearly half of all lake samples, varied widely among populations, from >20% of the sample (four lakes) to <5% of the sample (two lakes). These results suggest either that the rate of origin of >3x individuals differs among populations and/or that they represent lineages that can become common in some locations but not others. All polyploid males and >3x individuals had cytochrome-b haplotypes from clades or lineages that were nearly always or only found in triploid individuals, and none had haplotypes found otherwise only in diploids, indicating that they likely originated from triploids. Finally, we found that there was a strong positive correlation between variance in diploid genome and large variance in triploid genome sizes, suggesting that the processes that determine variance in genome size might operate on a very local scale (Burton and Husband 1999; Šmarda and Bureš 2006). These previously-undocumented patterns were revealed in a very large-scale study involving nearly 1000 individuals and over twenty populations, highlighting the value of broad, ecologically representative sampling strategies in uncovering relatively rare but potentially very important phenomena even in well-studied systems.

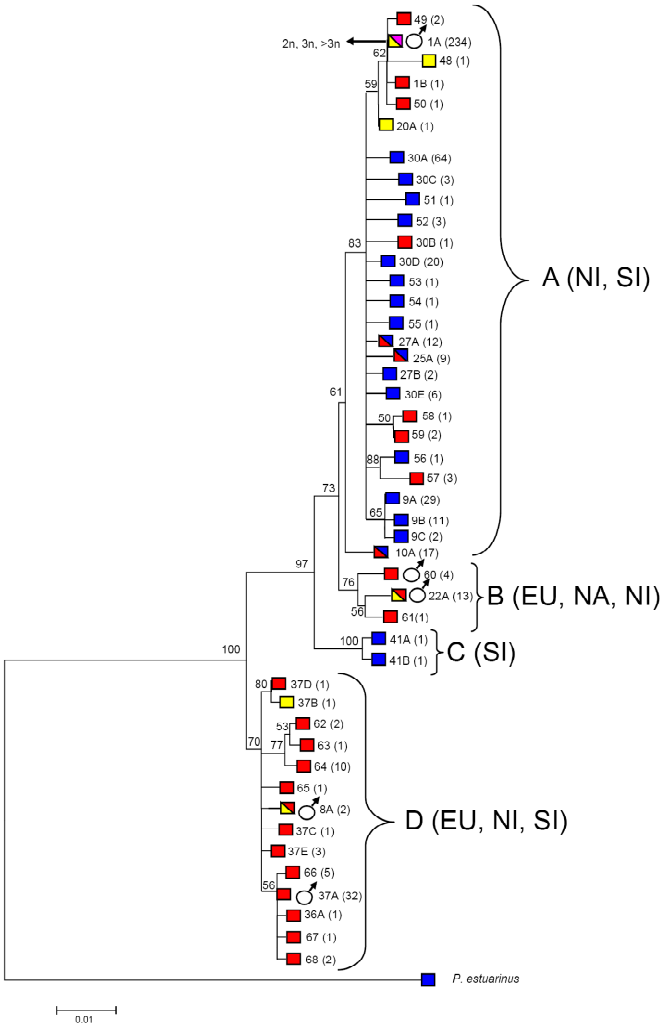


Figure 5. Neighbor-joining tree representing the 45 different cytochrome-b haplotypes and four major clades (A-D) detected in our survey. These clades were defined by at least 1.5% sequence divergence from all other clades and bootstrap support >70%. The two-letter acronym following the clade letter indicates where these snails or their ancestors were collected: "EU" = Europe, "NA" = North America, "NI" = North Island of New Zealand, and "SI" = South Island of New Zealand. The numbers to the left of nodes indicate bootstrap support; only values >50% are reported. The numbers to the right of boxes indicate the haplotype ID number, and the number in the parentheses to the right of the haplotype ID number indicates the number of individuals

represented by each haplotype. Haplotype ID numbers that include a letter indicate haplotypes that contained a 431 bp segment that was identical to a haplotype reported in Neiman and Lively (2004). These haplotypes were assigned the number of that 431 bp haplotype, while letters delineate haplotypes sharing a particular 431 bp haplotype that are different from one another at nucleotides outside of this 431 bp segment. Haplotype ID numbers without letters indicate haplotypes were novel with respect to any previously described *P. antipodarum* cytochrome-b haplotype. The color of the box at the end of each branch indicates whether haplotypes were found only in 2x individuals (blue), 3x individuals (red), or >3x individuals (yellow). Boxes with two colors indicate that the haplotype was represented by individuals of two ploidy levels. The box with both pink and yellow (indicated with arrow) had 2x, 3x, and >3x individuals. Male symbols next to a branch indicate that the lineage was also represented by polyploid males.

These findings bring up many questions that will require more experimental and direct approaches to answer fully. For one, why is there such wide variation in the relative frequency of >3x individuals across lake populations? The answer to this question will likely depend at least in part on the balance between the rate of transition to higher ploidy levels (i.e., rate of input of new >3x lineages) and the strength and direction of selection (or lack thereof) for or against polyploids (e.g., D'Souza et al. 2005, D'Souza and Michiels 2010).

A related and equally important question is the source of the ploidy elevation in *P. antipodarum*. Two of the most likely scenarios for the formation of natural autopolyploids were outlined by Ramsey and Schemske (1998) and Husband (2004): 1) the union of two unreduced gametes produced by the same individual (e.g., two 2x gametes produced by a diploid female), and 2) the union of gametes produced by a triploid with gametes from another individual ("triploid bridge"). There seems to be a consensus that the latter is likely to be far more common in nature, since it only requires one chromosome doubling event (Harlan and DeWet 1975; Husband 2004).

The presence of high relative frequencies of >3x *P. antipodarum* in populations where we did not find any diploids does suggest that it is unlikely that they usually originate from a doubling of the diploid genome. Autopolyploidization within diploids would also mean that >3x individuals should often carry the common mtDNA haplotypes of the diploid lineages. Instead, we found that these individuals harbored haplotypes nearly always associated with triploids, including several >3x individuals with haplotypes from well-supported clades in which no diploid individuals were found. These patterns suggest instead that triploid females are a more likely maternal source for these individuals, though we cannot at this time rule out a role for diploid females.

Triploid asexual female *P. antipodarum* might produce offspring with different ploidy levels or genome sizes than themselves either via nondisjunction during oogenesis and/or through fertilization by a male. The likelihood that variation in ploidy of asexual lineages originates from occasional fertilization of asexual females by sympatric males was emphasized by Maynard Smith (1978) and Lynch (1984). Empirical evidence for this phenomenon has since been reported in a variety of animal taxa (reviewed in Beukeboom and Vrijenhoek 1998), from flatworms (D'Souza et al. 2004; reviewed in D'Souza and Michiels 2010) and weevils (Tomiuk and Loeschcke 1992) to vertebrates such as *Poecilia formosa*, the Amazon molly (Avise 2008; Lampert et al. 2008). Our documentation of polyploid males and

ploidy variation even in lake samples with no diploid individuals, the possibility of production of haploid- and diploid- class sperm by polyploid males, and the observations of active copulatory behavior in asexual female *P. antipodarum* (Neiman and Lively 2005) suggest that this scenario cannot be rejected.

That polyploid males might be the source of fertilization leading to ploidy elevation at least in some cases is also consistent with our finding that both polyploid *P. antipodarum* individuals as well as putative sperm nuclei from polyploid males vary widely in nuclear DNA content. More broadly, we detected substantial among-population variation in the nuclear DNA content of diploid and triploid *P. antipodarum*, suggesting the presence of substantial intraspecific genome size variation. Similar variation has been detected in plants (e.g., Burton and Husband 1999; Sharbel and Mitchell-Olds 2001; Šmarda and Bureš 2006) and at least one animal system (Bosco et al. 2007). The within-population variance in genome size for sympatric diploids and triploids was positively correlated, as expected if triploid *P. antipodarum* originate from local diploid individuals (also see Dybdahl and Lively 1995; Neiman and Lively 2004). Extensive intraspecific variation in genome size and ploidy level has been documented in several plant taxa (e.g., Cires et al. 2008), but only rare reports of such variation exist from animals (e.g., Bosco et al. 2007; Lunt 2008). Why this is the case is not yet clear, but is probably linked to the fact that there has been much more effort devoted to the study of genome size and ploidy variation in plants than in animals (Mable 2004).

Regardless of its source, variation in nuclear DNA content across asexual lineages and within and across asexual subpopulations of *P. antipodarum* points to variation in genome size, which itself might be caused either by variation in chromosome size and/or number (e.g., Henry et al. 2005; Šmarda et al. 2008). Wide intraspecific variation in chromosome number has been documented in pulmonate snails (Husted and Burch 1946), root-knot nematodes (Lunt 2008), and in many triploid plants, which often make viable aneuploid offspring that vary widely in nuclear DNA content (Ramsey and Schemske 2002; Henry et al. 2005, 2010; reviewed in Comai 2005). These differences in chromosome number or size could provide a source of genetic variation to asexual lineages (Castageno-Serrano 2006). Since genome size, aneuploid karyotype, and ploidy level affect fitness in many other organisms (e.g., D'Souza et al. 2005; Gerstein and Otto 2009; Ching et al. 2010; Henry et al. 2010; Pavelka et al. 2010; Šmarda et al. 2008, 2010), variation in any of these could generate variation in fitness-related traits in *P. antipodarum*. Future studies should focus on characterizing the extent to which

ploidy level and genome size variation in *P. antipodarum* is related to the genetic diversity of asexual assemblages and how and whether such variation influences fitness-related traits in *P. antipodarum*.

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**Literature Cited**

Alves, M. J., M. M. Coelho, and M. J. Collares-Pereira. 2001. Evolution in action through hybridisation and polyploidy in an Iberian freshwater fish: a genetic review. Genetica, 111: 375-385.

Astaurov, B. L. 1969. Experimental polyploidy in animals. *Ann. Rev. Genet.* 3: 99-126.

Avise, J. C. 2008. Clonality: The genetics, ecology, and evolution of sexual abstinence in vertebrate animals. Oxford Univ. Press, New York, NY.

Bell, G. 1982. The masterpiece of nature. Croon Helm, London, UK.

Belshaw, R., D. L. J. Quicke, W. Volkl, and H. C. J. Godfray. 1999. Molecular markers indicate rare sex in a predominantly asexual parasitoid wasp. Evolution 53: 1189-1199.

Beukeboom, L. W., and R. C. Vrijenhoek. 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. J. Evol. Biol. 11: 755-782.

Bierzychudek, P. 1985. Patterns in plant parthenogenesis. Experientia 41: 1255-1264.

Bogart, J. P., and L. E. Licht. 1986. Reproduction and the origin of polyploids in hybrid salamanders of the genus *Ambystoma*. Can. J. Genet. Cytol. 28: 605-617.

Bosco, G., P. Campbell, J. T. Leiva-Neto, and T. A. Markow. 2007. Analysis of *Drosophila* species genome size and satellite DNA content reveals significant differences among strains as well as between species. Genetics 177: 1277-1290.

Castagnone-Sereno, P. 2006. Genetic variability and adaptive evolution in parthenogenetic root- knot nematodes. Heredity 96: 282-289.

Chaplin, J. A., and P. D. N. Hebert. 1997. *Cyprinotus incongruens* (Ostracoda): An ancient asexual? Mol. Ecol. 6: 155-168.

Christiansen, D. G., and H.-U. Reyer. 2009. From clonal to sexual hybrids: Genetic recombination via triploids in all-hybrid populations of water frogs. Evolution 63: 1754-1768.

Cires, E., C. Cuesta, M. A. Revilla, and J. A. F. Prieto. 2010. Intraspecific genome size variationand morphological differentiation of *Ranunculus parnassifolius* (Ranunculaceae), an Alpine–Pyrenean–Cantabrian polyploid group. Biol. J. Linn. Soc. 101: 251-271.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet. 6: 836- 846.

D'Souza, T. G., and N. K. Michiels. 2009. Sex in parthenogenetic planarians: Phylogenetic relic or evolutionary resurrection? In: Lost Sex (I. Schön, K. Martens, and P. Van Dijk, eds.),pp. 377-397, Springer, Amsterdam, Netherlands.

D'Souza, T. G., and N. K. Michiels. 2010. The costs and benefits of occasional sex: Theoretical predictions and a case study. J. Hered. 101: S34-41.

D'Souza, T. G., M. Storhas, H. Schulenburg, L. W. Beukeboom, and N. K. Michiels. 2004. Occasional sex in an 'asexual' polyploid hermaphrodite. Proc. Roy. Soc. Lond. B. 271: 1001-1007.

D'Souza, T. G., M. Storhas, and N. K. Michiels. 2005. The effect of ploidy level on fitness in parthenogenetic flatworms. Biol. J. Linn. Soc. 85: 191-198.

Dybdahl, M. F., and C. M. Lively. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail. J. Evol. Biol. 8: 385-398.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50: 1541-1548.

Gregory, T. R. 2005. The evolution of the genome. Elsevier, San Diego, USA.

Hall, T. A. 1999. BioEdit: a use-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuc. Acids Symp. Ser. 41: 95-98.

Harlan, J. R., and J. M. DeWet. 1975. On O. Winge and a prayer: the origins of polyploidy. Bot. Rev. 41: 361-390.

Henry, I. M., B. P. Dilkes, K. Young, B. Watson, H. Wu, and L. Comai. 2005. Aneuploidy and genetic variation in the *Arabidopsis thaliana* triploid response. Genetics 170: 1979-1988.

Henry, I. M., B. P. Dilkes, E. S. Miller, D. Burkart-Waco, and L. Comai. 2010. Phenotypic consequences of aneuploidy in *Arabidopsis thaliana*. Genetics 186: 1231-1245.

Husband, B. C. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. Biol. J. Linn. Soc. 82: 537-546.

Husted, L., and P. R. Burch. 1946. The chromosomes of polygyrid snails. Am. Nat. 80: 410-429.

Jokela, J., and C. M. Lively. 1995. Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution 49: 1268-1271.

Lampert, K. P., D. K. Lamatsch, P. Fischer, and M. Schartl. 2008. A tetraploid Amazon molly, *Poecilia formosa*. J. Hered. 99: 223-226.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328: 519-521.

Lively, C. M., and J. Jokela. 2002. Temporal and spatial distributions of parasites and sex in a freshwater snail. Evol. Ecol. Res. 4: 219-226.

Lunt, D. H. 2008. Genetic tests of ancient asexuality in Root Knot Nematodes reveal recent hybrid origins. BMC Evol. Biol. 8: 194.

Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes, and geographical parthenogenesis. Q. Rev. Biol. 59: 257-290.

Mable, B. K. 2004. 'Why polyploidy is rarer in animals than in plants:' myths and mechanisms. Biol. J. Linn. Soc. 82: 453-466.

Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, London, UK.

Neiman, M. and J. Jokela. 2010. Sex: Advantage. Encyclopedia of Life Sciences. John Wiley & Sons, Ltd., Chichester, UK.

Neiman, M., and C. M. Lively. 2004. Pleistocene glaciation is implicated in the phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail. Mol. Ecol. 13: 3085-3098.

Neiman, M., and C. M. Lively. 2005. Male New Zealand (*Potamopyrgus antipodarum*) persist in copulating with asexual and parasitically castrating females. Am. Mid. Nat. 154: 88-96.

Neiman, M., J. Jokela, and C. M. Lively. 2005. Variation in asexual lineage age in *Potamopyrgus antipodarum*, a New Zealand snail. Evolution 59:1945-1952.

Neiman, M., K. Theisen, M. E. Mayry, and A. D. Kay. 2009. Can phosphorus limitation contribute to the maintenance of sex? A test of a key assumption. J. Evol. Biol. 22: 1359-1363.

Normark, B. B. 1999. Evolution in a putatively ancient asexual aphid lineage: recombination and rapid karyotype change. Evolution 53: 1458-1469.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Ann. Rev. Genet. 34:401-437.

Pavelka, N., G. Rancati, J. Zhu, W. D. Bradford, A. Saraf, L. Florens, B. W. Sanderson, G. L. Hattem, and R. Li. 2010. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. Nature 468: 321-325.

Phillips, N. R., and D. M. Lambert. 1989. Genetics of *Potamopyrgus antipodarum* (Gastropoda:Prosobranchia): evidence for reproductive modes. NZ J. Zool. 16: 435-445.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14: 817-818.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. Ann. Rev. Ecol. Syst. 33: 589-639.

Roy, B. A. 1995. The breeding systems of six species of *Arabis* (Brassicaceae). Oecologia 95: 533-541.

Sahai, H., and M. M. Ojeda. 2005. Analysis of variance for random models. Birkhäuser, Boston, MA, USA.

Schultz, R. J. 1969. Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am. Nat. 103: 605-619.

Sharbel, T. F., and T. Mitchell-Olds. 2001. Recurrent polyploid origins and chloroplast phylogeography in the *Arabis holboellii* complex (Brassicaceae). Heredity 87: 59-68.

Šmarda, P., and P. Bureš. 2006. Intraspecific DNA content variability in *Festuca pallens* on different geographic scales and ploidy levels. Ann. Bot. 98: 665-678.

Šmarda, P., P. Bureš, L. Horová, and O. Rotreklová. 2008. Intrapopulation genome size dynamics in *Festuca pallens*. Ann. Bot. 102: 599-607.

Šmarda, P., L. Horová, P. Bureš, I. Hralová, and M. Marková. 2010. Stabilizing selection on genome size in a population of *Festuca pallens* under conditions of intensive intraspecificcompetition. New Phytol. 187: 1195-1204.

SPSS Inc. 2009. PASW Statistics 17.0, SPSS Inc., Chicago, IL, USA.

Stöck, M., D. K. Lamatsch, C. Steinlien, J. T. Epplen, W.-R. Grosse, R. Hock, T. Klapperstück, K. P. Lampert, U. Scheer, M. Schmid, and M. Schartl. 2002. A bisexually reproducing all-triploid vertebrate. Nat. Genet. 30: 325-328.

Sunnucks, P., P. R. England, A. C. Taylor, and D. F. Hales. 1996. Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia. Genetics 144: 747-756

Suomalainen, E. 1987. Polyploidy in association with parthenogenesis. In: Cytology and evolution in parthenogenesis (E. Suomalainen, A. Saura, and J. Lokki, eds.), pp. 71-112, CRC Press, Boca Raton, FL, USA.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Tomiuk, J., and V. Loeschcke. 1992. Evolution of parthenogenesis in the *Otiorhynchus scaber* complex. Heredity 68: 391-398.

van Dijk, P. J. 2003. Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. Phil. Trans. Roy. Soc. Lond. B. 358: 1113-1120.

Wallace, C. 1992. Parthenogenesis, sex, and chromosomes in *Potamopyrgus*. J. Moll. Stud. 58: 93-107.

Winterbourn, M. J. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). Malacologia 10: 283-321.

# Chapter II

## Discordance between nuclear and mitochondrial genomes in sexual and asexual lineages of the freshwater snail *Potamopyrgus antipodarum*

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**Abstract**

The presence and extent of mitonuclear discordance in coexisting sexual and asexual lineages provides insight into 1) how and when asexual lineages emerged, and 2) the spatial and temporal scales at which the ecological and evolutionary processes influencing the evolution of sexual and asexual reproduction occur. Here, we used nuclear SNP markers and a mitochondrial gene to characterize phylogeographic structure and the extent of mitonuclear discordance in *Potamopyrgus antipodarum*. This New Zealand freshwater snail is often used to study the evolution and maintenance of sex because obligately sexual and obligately asexual individuals often coexist. While our data indicate that sexual and asexual *P. antipodarum* sampled from the same lake population are often genetically similar, suggesting recent origin of these asexuals from sympatric sexual *P. antipodarum*, we also found significantly more population structure in sexuals versus asexuals. This latter result suggests that some asexual lineages originated in other lakes and/or in the relatively distant past. When comparing mitochondrial and nuclear population genetic structure, we discovered that one mitochondrial haplotype ("1A") was rare in sexuals but common and widespread in asexuals. Haplotype 1A frequency and nuclear genetic diversity were not associated, suggesting that the commonness of this haplotype cannot be attributed entirely to genetic drift and pointing instead to a role for selection.

## Introduction

Both mitochondrial and nuclear genomes carry genetic information that allows reconstruction of phylogeographic relationships. The phylogenetic information provided by these two genomes is often in agreement (Avise 1994), but it is increasingly evident that mitonuclear discordance, defined as a significant difference in the patterns of differentiation between these two marker types (Toews & Brelsford 2012), is also common (reviewed in: Funk & Omland 2003; Toews & Brelsford 2012). Research aimed at revealing the source of this mitonuclear discordance has implicated mechanisms such as incomplete lineage sorting, differential selection on nuclear and mitochondrial genomes, and asymmetrical hybridization. The latter is linked to phenomena like sex-biased dispersal, human-facilitated movement, and *Wolbachia* infection (which can cause mating incompatibilities between infected and uninfected hosts) (Toews & Brelsford 2012).

Many of these sources of mitonuclear discordance are connected to or directly caused by canonical sexual reproduction or by more unusual forms of genetic exchange (reviewed in: Schurko *et al.* 2009; Toews & Brelsford 2012). As such, the extent of mitonuclear discordance in asexuals can illuminate four areas of fundamental importance to researchers addressing why sexual reproduction is so common in nature: 1) how new asexual lineages are generated (Neiman & Schwander 2011), 2) their rate of origin (Burt 2000; Neiman *et al.* 2009), 3) how long these lineages persist (Neiman *et al.* 2009), and 4), the extent to which asexual lineages might experience occasional genetic exchange (Schurko *et al.* 2009).

Here, we used nuclear SNP markers and mitochondrial sequence data to build a comprehensive picture of population genetic structure and evaluate whether mitonuclear discordance is present in sexual and asexual lineages of *Potamopyrgus antipodarum*. This New Zealand freshwater snail has been used as a model system for studying the maintenance and distribution of sexual reproduction in nature for over 25 years (e.g., Lively 1987; Jokela *et al.* 1997; Jokela *et al.* 2009). Sexual *P. antipodarum* are diploid and asexual *P. antipodarum* are typically polyploid (3x and >3x) (Wallace 1992; Neiman *et al.* 2011; 2012), though there is some evidence for the presence of diploid asexuals in two New Zealand lakes

(see Methods and Neiman *et al.* 2011). Both allozyme (Dybdahl & Lively 1995) and mitochondrial data (Neiman & Lively 2004; Neiman *et al.* 2011) indicate that unlike many asexuals (recently reviewed in: Kearney 2005; Neiman & Schwander 2011), asexual *P. antipodarum* are derived from sexual conspecifics rather than of hybrid origin. This latter point is important because it means that direct or indirect consequences of hybridization cannot account for differences between coexisting sexual and asexual individuals or subpopulations (Maynard Smith 1978; Kearney 2005; Jokela *et al.* 1997).

The nuclear (Dybdahl & Lively 1995) and mitochondrial datasets (Dybdahl & Lively 1995; Neiman & Lively 2004; Neiman *et al.* 2011) that had been used to test hypotheses of hybrid vs. non-hybrid origin in *P. antipodarum* were also used to address whether asexual *P. antipodarum*, which often coexist with sexual *P. antipodarum* (Lively 1987), were of recent local origin (recently derived from sympatric sexuals) vs. non-local and/or non-recent origin (derived from allopatric sexuals and/or from sympatric sexuals in the relatively distant past). Whether asexual organisms are recently derived from sympatric sexual relatives represents critical information for evaluating whether sexual populations are likely to face threats from ecologically and phenotypically similar asexual lineages (Case & Taper 1986).
Dybdahl and Lively (1995) genotyped *P. antipodarum* from four New Zealand lake populations at six allozyme loci and showed that asexual *P. antipodarum* were more closely related to sympatric sexuals than to allopatric sexuals or asexuals, suggesting local and recent origin of asexual lineages from sympatric sexual conspecifics. In contrast to these results, two geographically broader mitochondrial sequence-based phylogeographic studies found that a majority of asexual *P. antipodarum* sampled from many different lakes shared a single mitochondrial haplotype that was rare in sexuals (Neiman & Lively 2004; Neiman *et al.* 2011).

Uncovering the reason for the strikingly different patterns of genetic structure among asexual *P. antipodarum* revealed by nuclear (allozyme) and mtDNA data requires genotyping multiple sexual and asexual individuals from many populations at both nuclear and mitochondrial loci and then using these data to compare and contrast the population genetic structure that these markers reveal. The most straightforward potential outcome of this

approach would be broad mitonuclear concordance within asexual *P. antipodarum* such that asexuals are most similar to coexisting sexuals at both nuclear and mtDNA markers. This result would suggest recent origin of the asexuals from sympatric sexuals. Alternatively, a pattern where many asexuals harbor nuclear and/or mitochondrial genotypes that are different from coexisting sexuals will implicate a more complex scenario of asexual lineage origin, suggesting that at least some asexual lineages are widespread and of allopatric and/or non-recent derivation from sexual *P. antipodarum*.

Based on this logic, we generated nuclear SNP and mitochondrial sequence data from *P. antipodarum* from 16 New Zealand lake populations to compare and contrast the population genetic structure of sexual and asexual *P. antipodarum* as reflected by both the mitochondrial and nuclear genomes. In particular, we address 1) the extent to which asexual lineages of *P. antipodarum* are of recent origin from coexisting sexuals, 2) how mitonuclear discordance is distributed in sexual vs. asexual individuals, and 3) whether an especially common and widespread mitochondrial haplotype found primarily in asexual lineages is associated with common and widespread nuclear genotypes.

## Materials and methods

### *Sample collections*

We collected 574 snails in 10 New Zealand lakes in January 2009 and 6 New Zealand lakes in January 2010 (Table 1, Fig. 2) by pushing kick nets through vegetation and washing snails off rocks. All samples were collected in the shallow (0-1 m) littoral area of the lakes with the exception of Lake Alexandrina, where the mid-water (1-3 m) and deep-water (4-6 m) habitats were also sampled using snorkeling equipment. Shallow and deep habitats in this well-studied lake are known to differ in the relative frequencies of sexual and asexual *P. antipodarum*, with sexual diploids most common in the shallow and asexual triploids dominating the deep; the mid-water habitat harbors intermediate proportions of sexuals and asexuals (Fox *et al.* 1996). Including samples from the deeper habitats in Lake Alexandrina is unlikely to bias the across-lake results, as there are no significant differences in genetic similarity between sexual

and asexual snails sampled from different habitats in this lake (D. Paczesniak, unpublished data). All snails were transported to the University of Iowa (2009) or the Swiss Federal Institute of Aquatic Science and Technology (Eawag) (2010) and housed in aquaria until further analysis (up to 4 weeks, Iowa; up to 8 weeks, Eawag). Individual snails were dissected under a dissecting microscope and sexed (males were distinguished from females by a presence of a penis). The head was split in two halves and either snap-frozen in separate tubes for DNA extraction and flow cytometry (Iowa) or used immediately for flow cytometry analysis (Eawag).

*Flow cytometry*

The intensity of fluorescence of cell nuclei extracted from snail heads and stained with DAPI (4',6-Diamidino-2-Phenylindole) relative to a size standard were used to assign ploidy level and thus reproductive mode (2x individuals are sexual, 3x and >3x individuals are asexual: Wallace 1992; Neiman *et al.* 2011; 2012). The only potential exceptions to the diploid-sexual association were made for diploids from lakes Okareka and Te Anau. We treated these snails differently because no diploid males have ever been collected from these lakes, suggesting that diploid females from Okareka and Te Anau may be in fact asexual (Neiman *et al.* 2011). Because the confirmation of asexual status of the diploids in these two lakes requires additional study, we excluded Okareka and Te Anau diploids from the sexual group in the two analyses in which we directly compared sexual and asexual snails (testing the influence of reproductive mode on the assignment of individuals to their population of origin by the clustering analysis and testing the association of 1A haplotype with asexuality). Samples collected in 2009 were analyzed at the University of Iowa and those collected in 2010 at Eawag. The detailed laboratory protocols, methods of data analysis, and methods used to combine the data obtained in the two different laboratories are presented in Neiman *et al.* (2011). Altogether, we were able to successfully assign ploidy in 504 of the 532 snails (94.7%) for which we obtained a SNP genotype (see below).

Table1. Characteristics of the 16 sampled New Zealand lakes. Sample sizes (N) include only individuals for which we obtained a SNP genotype with not more than 11 missing loci (92.7 % of sampled individuals; see Methods section "*SNP marker development and genotyping*" for details.

| Population (acronym) | Geographic coordinates (Latitude, Longitude) | year collected | N | N diploids | Percentage 2x | Percentage 2x males | N unique genotypes among diploids | Percentage 3x | Percentage >3x | Percentage unknown ploidy | N unique lineages among polyploids | Haplotypes (percentage among individuals) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alexandrina (AX) | -43.937784, 170.455286 | 2009 | 122 | 50 | 40.98 | 13.11 | 49 | 59 02 | 0 00 | 0 00 | 40 | 10A (15.6); 10A+1(B) (1.1); 1A (45.6); 25A (8.9); 30A (16.7); 30A+1(B) (1.1); 30C (4.4); 30E (5.6); 50 (1.1) |
| Clearwater (CW) | -43.606176, 171.046179 | 2009 | 31 | 22 | 70.97 | 25.81 | 22 | 22 58 | 9.68 | 0 00 | 6 | 1A (21.4); 30A (75 0); 30A+1(A) (3.6) |
| Grasmere (GR) | -43.061572, 171.774601 | 2009 | 28 | 7 | 25.00 | 14.29 | 7 | 64 29 | 7.14 | 0 00 | 19 | 1A (73.1); 30A (26 9) |
| Gunn (GU) | -44.875886, 168.090282 | 2010 | 32 | 0 | 0 00 | NA | NA | 71 88 | 28.13 | 0 00 | 21 | 10A (14.3); 10A+1(A) (4.8); 10A+2(A) (9.5); 1A (23 8); 1A+1(A) (4 8); 20A (33 3); 37A (4 8); 68 (4 8) |
| Haupiri (HR) | -42.569563, 171.688418 | 2010 | 37 | 3 | 8.11 | 8.11 | 3 | 83.78 | 8.11 | 0 00 | 22 | 1A (88 0); 27A (12 0) |
| Heron (HE) | -43.478353, 171.172282 | 2009 | 38 | 30 | 78.95 | 23.68 | 30 | 15.79 | 5 26 | 0 00 | 7 | 1A (24 3); 30A (67.6); 52 (8.1) |
| Ianthe (IA) | -43.053442, 170.624366 | 2010 | 31 | 29 | 93.55 | 41.94 | 28 | 6.45 | 0 00 | 0 00 | 2 | 1A (3.2); 37A (3.2); 41A/B+1(A) (6.5); 41A/B+2(A) (9.7); 41A/B+2(B) (12.9); 41A/B+3(B) (3.2); 41A/B+4(A) (3 2); 41B (54.8); 41B+1(A) |
| Mapourika (MP) | -43.31801, 170.204244 | 2010 | 33 | 7 | 21.21 | 3.03 | 7 | 24 24 | 0 00 | 54.55 | 5 | 1A (34 5); 1A+1(B) (6.9); 27A (37.9); 30D (17.2); 30D+1(A) |
| Okareka (OK) | -38.171413, 176.361585 | 2009 | 13 | 5 | 38.46 | 0.00 | 4 | 0.00 | 0 00 | 61.54 | 0 | 37A (20.0); 61 (10.0); 62/64+1(A) (10.0); 68 (60.0) |
| Poerua (PR) | -42.704813, 171.495172 | 2009 | 16 | 1 | 6 25 | 0.00 | 1 | 56 25 | 37.50 | 0 00 | 8 | 1A (100.0) |
| Rotoiti (RT) | -41.820374, 172.837135 | 2009 | 18 | 0 | 0 00 | NA | NA | 27.78 | 72.22 | 0 00 | 12 | 1A (91.7); 37B (8.3) |
| Selfe (SE) | -43.240251, 171.519869 | 2009 | 33 | 16 | 48.48 | 24.24 | 16 | 51 52 | 0 00 | 0 00 | 13 | 1A (44 8); 27A (37 9); 27B (3.4); 9A (13.8) |
| South Mavora (MV) | -45.302545, 168.173651 | 2010 | 35 | 0 | 0 00 | NA | NA | 77.14 | 22.86 | 0 00 | 20 | 10A+1(A) (20.0), 1A (35.0); 1A+1(C) (5.0); 20A (25.0); 20A+1(A) (10.0); 20A+2(A) (5 0) |
| Taupo (TP) | -38.806781, 175.906716 | 2009 | 17 | 0 | 0 00 | NA | NA | 100.00 | 0.00 | 0 00 | 13 | 20A (7.7); 27A (7.7); 37A (23.1); 37E (7.7); 64 (30.8); 64+1(A) (7.7); 65 (7.7); 66 (7.7) |
| Te Anau (TA) | -45.201264, 167.765052 | 2010 | 35 | 9 | 25.71 | 0.00 | 4 | 74 29 | 0 00 | 0 00 | 21 | 10A (20.0); 10A+1(A) (50.0); 10A+2(B) (6.7); 10A+2(C) (3 3); 10A+2(D) (3 3); 10A+4(A) (10.0); 10A+6(A) (3 3); 10A+6(B)(3 3) |
| Waikaremoana (WK) | -38.774538, 177.112356 | 2009 | 13 | 0 | 0 00 | NA | NA | 76 92 | 7.69 | 15.38 | 10 | 22A (41.7); 37A (25.0); 57 (8 3); 58 (8.3); 59 (8.3); 60 (8 3) |

*DNA extraction*

We extracted DNA from snail head tissue with the Qiagen DNeasy Plant kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol with one modification: we used nanopure water rather than the elution buffer for the final dilution.

*SNP marker development and genotyping*

For the purpose of this study we used the *P. antipodarum* transcriptome (Wilton *et al.* 2013) to develop a set of nuclear SNP (Single Nucleotide Polymorphism) markers. We used two different strategies to isolate two different sets of contigs from the transcriptome for candidate SNP detection and selection:

1) We used contigs that returned a top hit to molluscs in a blastx search (Altschul *et al.* 1997) in GenBank. This strategy ensured that we did not use SNPs within sequences generated from microorganisms present on or in the *P. antipodarum* used for the transcriptome. Next, we used blastx to further exclude contigs with hits to gene families or gene isoforms in molluscs, which reduces the possibility of including members of multi-gene families.

2) We used contigs that matched single-gene families identified by Holton & Pisani (2010), who used metazoan genome data and identified single-gene families suitable for phylogeny reconstruction.

Within these two sets of contigs, we then identified variable nucleotide sites that represented candidate SNP sites. Next, we used blastx to assign reading frame and thus determine whether each putative SNP comprised a synonymous or non-synonymous change. Because we wanted to focus on neutral (or nearly neutral) sites, we selected only synonymous SNPs. In the case of multiple SNPs per contig that satisfied our criteria, the target SNP was chosen randomly. Sequences of 100 candidate SNPs along with 100-500 bp of the flanking sequence on either side were sent to the Genomic Technologies Facility at Iowa State University for design of the Mass Array® iPLEX® SNP genotyping assay and for the SNP genotyping itself. Out of the genotyping multiplex assays designed *in silico*, we chose the assay with the highest number of loci (32). Of the 32 genotyped loci, 23 were polymorphic and scorable in >70% of

individuals genotyped; only these loci were used for further analysis. Out of the 574 genotyped individuals we excluded from further analysis 42 individuals with missing data at 12 or more loci, leaving our final dataset with 532 individuals. For individuals of all ploidy levels, the genotypes at each locus were scored as homozygous when only one allele was present and as heterozygous when two alleles were present. It was not possible to reliably assign the identity of additional alleles in polyploid individuals (e.g., to distinguish between genotypes AAB and ABB).

In order to identify members of the same asexual lineage, we then used these multilocus SNP genotypes and GenoType software (Meirmans & Van Tienderen 2004) to assign genotypic identity to polyploid individuals. Missing data were not considered, and the threshold for assigning individuals as members of different lineages was set to zero, meaning that individuals with pairwise distances between genotypes larger than zero were scored as members of a different lineage. If individuals with identical SNP genotypes had different mitochondrial haplotypes or were of different ploidy level, they were manually assigned as members of a unique lineage. To account for the possibility that there may be rare diploid asexual individuals, we also identified identical SNP genotypes among diploid individuals in our samples using GenoType software with the same settings as described above.

*Mitochondrial sequencing*

A 718-bp portion of the mitochondrial cytochrome-b gene had already been sequenced in 243 of the 532 *P. antipodarum* for which we had generated SNP genotypes (Neiman *et al.* 2011). We followed protocols used in Neiman *et al.* (2011) to sequence the same 718 bp segment in the additional 289 *P. antipodarum* included in the present study.
The newly acquired cytochrome-b sequences were edited and aligned in BioEdit (Hall 1999). We then trimmed the sequences, where possible, to the same 718 bp fragment of the gene used in Neiman *et al.* (2011). Next, each new sequence was used as a query in a blastn search against NCBI's nucleotide database to determine whether the sequence was identical to one of the 45 different haplotypes identified in Neiman *et al.* (2011) (Genbank accession #s JF518834-JF518877). If so, the haplotype was assigned the same name as the matching

Genbank haplotype. If not, the haplotype was given the name of the closest match on Genbank plus the number of substitutions separating it from that haplotype.
After assigning the haplotype names, we trimmed the sequences to the 634 bases that were reliably amplified in all individuals and then used this dataset in all subsequent analyses. To visualize the genetic structure of mitochondrial sequences, we constructed a maximum-parsimony haplotype network at the 95% connection limit using TCS version 1.21 (Clement *et al.* 2000). Next, we used BIC value comparison to select the model of sequence evolution that best fit the data. We then ran 1000 replicates of neighbour-joining and maximum-likelihood bootstrap procedures under the best-fitting model (the Tamura 3-parameter model: rate variation among sites was modelled using gamma distribution, shape parameter = 0.5066) to evaluate the statistical support of the resulting tree topologies. Both model selection and tree construction were performed using MEGA 5 (Tamura *et al.* 2011).

*Genetic structure: mitochondrial and nuclear data*

We first confined our analyses to the 414 unique genotype/ploidy/mitochondrial haplotype combinations in order to avoid biases arising from the treatment of genetically identical individuals (i.e., members of the same asexual lineage) as independent data points. Next, we used calculations of pairwise $F_{st}$ values for the five populations for which we had a sufficient sample of sexual individuals to estimate F statistics (N>16 individuals; Alexandrina, Clearwater, Heron, Ianthe, and Selfe) to evaluate the ability of our SNP markers to detect genetic structure and to quantify differentiation between sexual populations. We also calculated the unbiased estimate of global $F_{st}$ ($\theta$, Weir & Cockerham 1984) and bootstrap 95% confidence limits using Fstat version 2.9.3 (Goudet 1995).
We used Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.* 2010), as implemented in the R package *adegenet* (Jombart 2008; R Development Core Team 2009), to cluster genetically similar individuals according to their multilocus SNP genotypes. This method first transforms the data using principal components analysis, which ensures that the variables are not correlated and the number of variables is smaller than the number of individuals. Then discriminant analysis partitions the variance into among- and within-group components, maximizing separation between groups. Because DAPC does not assume any

underlying population genetic model, it is applicable to a mixed sample of sexual and asexual individuals (Jombart *et al.* 2010).

We performed the DAPC analysis on the nuclear SNP dataset using two sets of priors: 1) population of origin (16 lakes) and 2) mitochondrial haplotype group (7 groups, Fig. 2). We defined these groups as those sets of haplotypes separated from one another by at least 5 mutational steps (>1% pairwise divergence). Because the largest of these groups contained two distinct sets of haplotypes that were clearly differentiated on the basis of high relative frequency of sexuals (P3, Fig. 2) versus asexuals (P5, Fig. 2), we treated these subgroups as separate groups for this analysis. The inclusion of these separate P3 and P5 groups allowed us to evaluate whether differences in nuclear population genetic structure were evident in haplotype groups that tend to differ in reproductive mode. Because group P5 also included a haplotype (1A) that is overwhelmingly common in South Island asexual *P. antipodarum*, this analysis also allowed us to address whether there was a specific nuclear population genetic structure associated with this haplotype. In order to compare the population genetic structure revealed by nuclear and mitochondrial data, we also performed the DAPC analysis on the mitochondrial haplotype data using population of origin as prior.

We performed these three DAPC analyses with these two types of priors because comparison of their ability to explain population genetic structure in sexuals versus asexuals can provide important information on the origin and diversity of asexual lineages. First, the population of origin represents both the present mating pool for sexuals and the present selection arena for asexuals. These processes are expected to generate population genetic structure unless countered by migration (asexuals) and/or gene flow (sexuals and possibly asexuals). Our analysis of among-lake $F_{st}$ values in sexual *P. antipodarum* revealed substantial differentiation and thus low gene flow among sexual populations, and also indicates that population of origin confers meaningful genetic information for sexuals (Table 2, also see Dybdahl & Lively 1996). The implications of this $F_{st}$ information are that we can use population of origin priors for the nuclear data to address whether population of origin also confers meaningful genetic information for asexual *P. antipodarum*. Given the evidence from our $F_{st}$ analyses that sexual *P. antipodarum* populations experience little gene flow (Table 2), we predict that if asexual *P. antipodarum* are of recent origin from sympatric sexuals, the ability of the population of origin prior to explain asexual nuclear population genetic structure

should be similar to or greater than the ability of this prior to explain structure in sexual *P. antipodarum*.

In contrast to population of origin, mitochondrial haplotype group membership reflects historical events (e.g., Pleistocene glaciation, Trewick *et al.* 2000; Neiman & Lively 2004) that occurred thousands to millions of years ago. This means that individuals that share a mitochondrial haplotype group - regardless of current population of origin - are likely to also share at least some of their evolutionary history. Inclusion of both population of origin and mitochondrial haplotype group priors thus gives us information on the evolutionary histories of sexual and asexual *P. antipodarum* across a wide timescale. We can also use comparisons between outcomes of the DAPC analyses with population of origin prior between the nuclear and mitochondrial datasets to provide information on the extent to which there is mitochondrial versus nuclear population genetic structure across populations and in diploids (all sexual with possible exception of snails from Te Anau and Okareka) versus asexual polyploids.

We can evaluate how well our data fit these various predictions by comparing our DAPC analyses results to a null prediction: if there was no structure in the data that can be explained by our priors, then each individual should have the same probability of being placed in any of the posterior groups (1/16 for the population prior, 1/7 for the haplotype group prior). Based on this logic, we compared the mean value of the maximum probability that an individual belonged to any of the posterior groups assigned by DAPC analysis to the null values of 1/16 (for analyses using population prior) and 1/7 (haplotype group prior) using 95% confidence intervals (obtained from 10000 bootstrap replicates). We analysed means of all individuals together and also conducted separate analyses for each ploidy level. We performed the DAPC analyses with the nuclear SNP data using the population of origin and haplotype group priors and with the mitochondrial haplotype data using the population of origin prior.

Finally, we used information from the DAPC analysis of the nuclear SNP data with the predefined population of origin prior to construct a contingency table evaluating whether and how often population of origin correctly predicted cluster membership (Fig. 6). We then used logit models to test whether a significantly higher proportion of sexual vs. asexual individuals was correctly reassigned to their prior groups (population of origin). In a logit model, the binomial dependent variable (correctly vs. incorrectly reassigned) can be explained with

categorical independent variables, which in our case were population of origin and reproductive mode (following, e.g., Murtaugh (1988), Norusis (1990), Jokela & Mutikainen (1995)). We used this logit analysis for the nine populations where both diploid sexuals and polyploid asexuals were present to evaluate whether correct vs. incorrect reassignment to prior group was independent of the reproductive mode and/or population (lake). The analyses were performed using IBM® SPSS Statistics version 19 and Microsoft® Excel 2010.

***Analyses of the common mitochondrial haplotype 1A***

*An association with asexuality?*

Our data revealed that 37 % (63 % of all unique polyploid genotypes and 5 % of unique diploid genotypes) of *P. antipodarum* in our study harbored a single recently derived mitochondrial haplotype (1A). We used logit models to test whether this pattern reflected significant association of the 1A haplotype with asexuality. As explained above, in a logit analysis, the binomial dependent variable (here, carrying the 1A haplotype versus any other haplotype) can be explained with categorical independent variables; here, population and reproductive mode. We used this logit analysis and the nine populations where both diploid sexuals and polyploid asexuals were present to evaluate whether the presence of 1A was independent of the population (lake) and/or reproductive mode. The analyses were performed using IBM® SPSS Statistics version 19 and Microsoft® Excel 2010.

*A role for selection?*

All else being equal, genetic diversity in the nuclear and mitochondrial genomes is expected to be proportional to the effective population size experienced during the recent evolutionary history of the genome (Gompert *et al.* 2008). The implications are that insight into why a particular haplotype is overrepresented can come from comparisons of the levels of nuclear and mitochondrial genetic diversity in populations with varying proportions of the haplotype in question. As any mitochondrial haplotype becomes relatively common in a population, mitochondrial genetic diversity will automatically become relatively low. Nuclear genetic diversity, however, will only decrease as the frequency of a particular mtDNA haplotype becomes common if similar evolutionary forces were affecting both genomes – e.g., a recent population bottleneck. Conversely, retention of high nuclear diversity in populations where

the haplotype is common (and mitochondrial diversity is low) is predicted under positive selection favouring a mitochondrial genome and/or cytoplasm bearing a particular mitochondrial haplotype (Gompert *et al.* 2008). By this logic, we used estimates of nuclear diversity in the study populations to determine whether relatively high frequency of the 1A haplotype (and thus relatively low mitochondrial diversity) is associated with reduction of genetic diversity in nuclear genomes.

As a measure of nuclear genetic diversity we used the mean of pairwise comparisons between individual genotypes with the "band-sharing" pairwise genetic identity measure that was developed by Tomiuk *et al.* (2009) to compare intraspecific genetic differentiation and that can be used for samples that vary in ploidy level. The mean of pairwise genetic identities between individual genotypes from the same lake provides an estimate of the genetic diversity of the population, with values closer to 1 indicating less diverse populations (individuals are more similar to each other) and values closer to 0 indicating more diverse populations (individuals are more dissimilar to each other). We calculated these pairwise genetic identity measures with POPDIST version 1.2.4 (Tomiuk *et al.* 2009).
We then used a linear regression model (as implemented within IBM® SPSS Statistics version 19) to examine the relationship between nuclear genetic diversity and the frequency of the 1A haplotype. This analysis allowed us to examine whether nuclear diversity was reduced in populations with relatively high frequency of the 1A haplotype (and thus relatively low mitochondrial diversity).

## Results

### *Genetic structure: mitochondrial and nuclear data*

We identified 52 unique mitochondrial haplotypes. The haplotype network at 95% connectivity separated these haplotypes into three networks (Fig. 1) that correspond to the major groups previously described in *P. antipodarum* (Neiman & Lively 2004; Neiman *et al.* 2011). Similar to these previous studies, we found that a single recently derived mitochondrial

haplotype (1A) was very common relative to other haplotypes, found in 36% of all individuals and in 12 out of 16 lakes (Table 1).
The same major divisions identified by the haplotype network procedure were also visible in the neighbour-joining tree (Fig. 2). The maximum likelihood method yielded the same tree topology with very similar bootstrap values (tree not shown).

The five samples for which we had the largest samples of diploid sexual genotypes (N>16) show substantial population differentiation (Table 2), with a mean $F_{st}$ of 0.29 and standard deviation of 0.11. The global $F_{st}$ estimate $\theta = 0.298$ (95% confidence limits: lower CL=0.203, upper CL=0.390) is somewhat higher than the $\theta = 0.128$ (95% confidence limits: lower CL=0.031, upper CL=0.225) estimated with allozyme data by Dybdahl & Lively (1996), which might be explained by the larger and more geographically widespread set of lakes we sampled.

Table 2. Pairwise $F_{st}$ for the 5 populations for which the number of unique sexual genotypes > 16.

| | Alexandrina | Clearwater | Heron | Ianthe | Selfe |
|---|---|---|---|---|---|
| Alexandrina | | | | | |
| Clearwater | 0.2231 | | | | |
| Heron | 0.2373 | 0.1076 | | | |
| Ianthe | 0.4061 | 0.4439 | 0.327 | | |
| Selfe | 0.2313 | 0.2611 | 0.2476 | 0.4103 | |

To visualize the phylogeographic structure of the nuclear data, we plotted the result of the DAPC analysis with population of origin as prior (Fig. 3). While there are no clear separations into non-overlapping groups, there is clustering of samples from the same geographic region. For example, the separation of samples is mostly along the first axis (LD1), which differentiates the east coast of the South Island from the other regions.

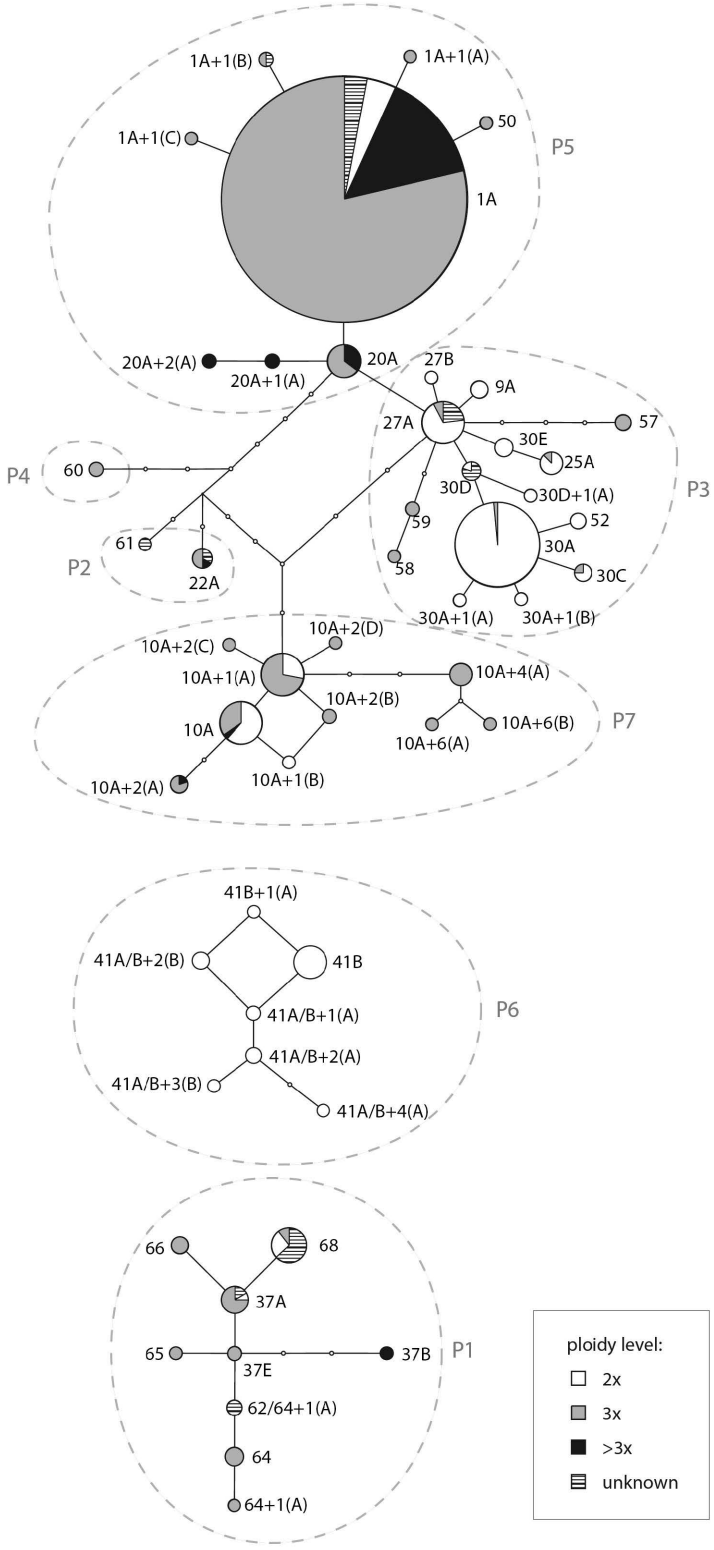
1A+1(B)
1A+1(A)
50
1A+1(C)
1A
P5
20A+2(A)
20A
20A+1(A)
27B
9A
27A
57
30E
25A
P4
60
30D
30D+1(A)
P3
59
52
61
30A
58
30C
22A
P2
30A+1(A)
30A+1(B)
10A+2(D)
10A+2(C)
10A+1(A)
10A+4(A)
10A+2(B)
10A
10A+6(B)
P7
10A+6(A)
10A+2(A)
10A+1(B)
41B+1(A)
41A/B+2(B)
41B
41A/B+1(A)
P6
41A/B+2(A)
41A/B+3(B)
41A/B+4(A)
66
68
37A
65
37B
P1
37E
62/64+1(A)
64
64+1(A)
ploidy level:
2x
3x
>3x
unknown

Fig. 1 (prev. page). Maximum-parsimony network of mitochondrial cytochrome-b haplotypes. The TCS analysis at the 95% connection limit identified three networks. The area of each pie chart is approximately proportional to the frequency of the haplotype, and the proportion of each of 4 types of shading represents the proportion of individuals with that haplotype represented by each of three possible ploidy levels as well as individuals with unknown ploidy. Each line segment represents a single nucleotide substitution, and the nodes represent unsampled haplotypes. P1-P7 (dashed lines) represent haplotype groups used in the DAPC analysis (see text for details).

We then compared the patterns of divergence revealed by the DAPC analyses using population of origin versus mitochondrial haplotype group as priors (Fig. 4). Lakes for which the two analysis outcomes are similar represent populations that have been isolated long enough that the lake of origin and mitochondrial haplogroup priors are similarly informative regarding DAPC cluster assignment (e.g., Te Anau, Ianthe). For South Island lakes, the DAPC analyses with population of origin and mitochondrial prior reveal a west coast/east coast division as well as fine-scale structure among lakes.

Next, we compared DAPC analysis outcomes for sexual diploids versus asexual polyploids (Fig. 4). While the asexuals are often quite similar to coexisting sexuals (e.g., Mapourika, Clearwater), differentiation between coexisting sexuals and asexuals is apparent in several lakes. This differentiation is especially notable for the analysis with the population of origin prior for samples from Alexandrina, Selfe and Grasmere, where many asexual individuals have nuclear genotypes that are not found in sympatric sexuals (Fig. 4, top panel).

We then used comparisons of the 95% confidence intervals around the mean maximum probability of membership in a DAPC-assigned cluster relative to the null assumption of membership probability if priors convey no useful information (equal across all clusters) to determine whether either or both of the population of origin and mitochondrial haplotype group priors provided useful information regarding genetic structure (Fig. 5).

While both priors explained significantly more structure than by chance alone (Fig. 5A), the population of origin prior was more than two times better at predicting cluster membership (13.1× relative to null) than the mitochondrial haplotype prior (5.2× relative to null). The population of origin prior also significantly exceeded null expectations for the mitochondrial haplotype data, but to a lesser extent (9.5× relative to null) than for the nuclear data.

The same analysis performed for the different ploidy levels showed significantly lower values of mean maximum cluster membership for both triploid and >3x individuals vs. diploid individuals with the population of origin prior and for both the nuclear and mitochondrial data, but we found no significant differences between triploid and >3x individuals for any of the comparisons (Fig. 5B). These results suggest that there is more population genetic structure for diploid *P. antipodarum,* which are all or nearly all sexual, than for polyploid asexual *P. antipodarum*. There were no significant differences between any of the pairwise comparisons of ploidy levels in the analysis using nuclear data with mitochondrial group prior, meaning that the extent of nuclear structure that can be explained by mitochondrial haplotype group does not differ between the different ploidy levels.

We then used logit models to ask if assignment of individuals to their population of origin differed between sexual and asexual snails. We first compared the fit of a model with main effects of lake of origin and reproductive mode to a full model including both main effects and the interaction term. Because the full model did not fit the data significantly better than the reduced model ($\chi^2$=15.38, *P*=0.052, df=8), suggesting that the amount of variation explained by the interaction term was not very high, we used the reduced model (main effects only) to evaluate the relative strength of reproductive mode vs. lake of origin as explanations for the correct assignment of *P. antipodarum* to their prior group (population of origin). We found that both lake ($\chi^2$=20.19, *P*=0.010, df=8) and especially reproductive mode ($\chi^2$=20.19, $P=7\times10^{-6}$, df=1) significantly explained the proportions of correctly reassigned individuals. In particular, a higher overall proportion of sexuals (82.2 %) than asexuals (61.5 %) were correctly assigned to their population of origin by the DAPC analysis. This result provides another line of evidence for a higher level of population genetic structure for diploid sexual vs. polyploid asexual *P. antipodarum.*

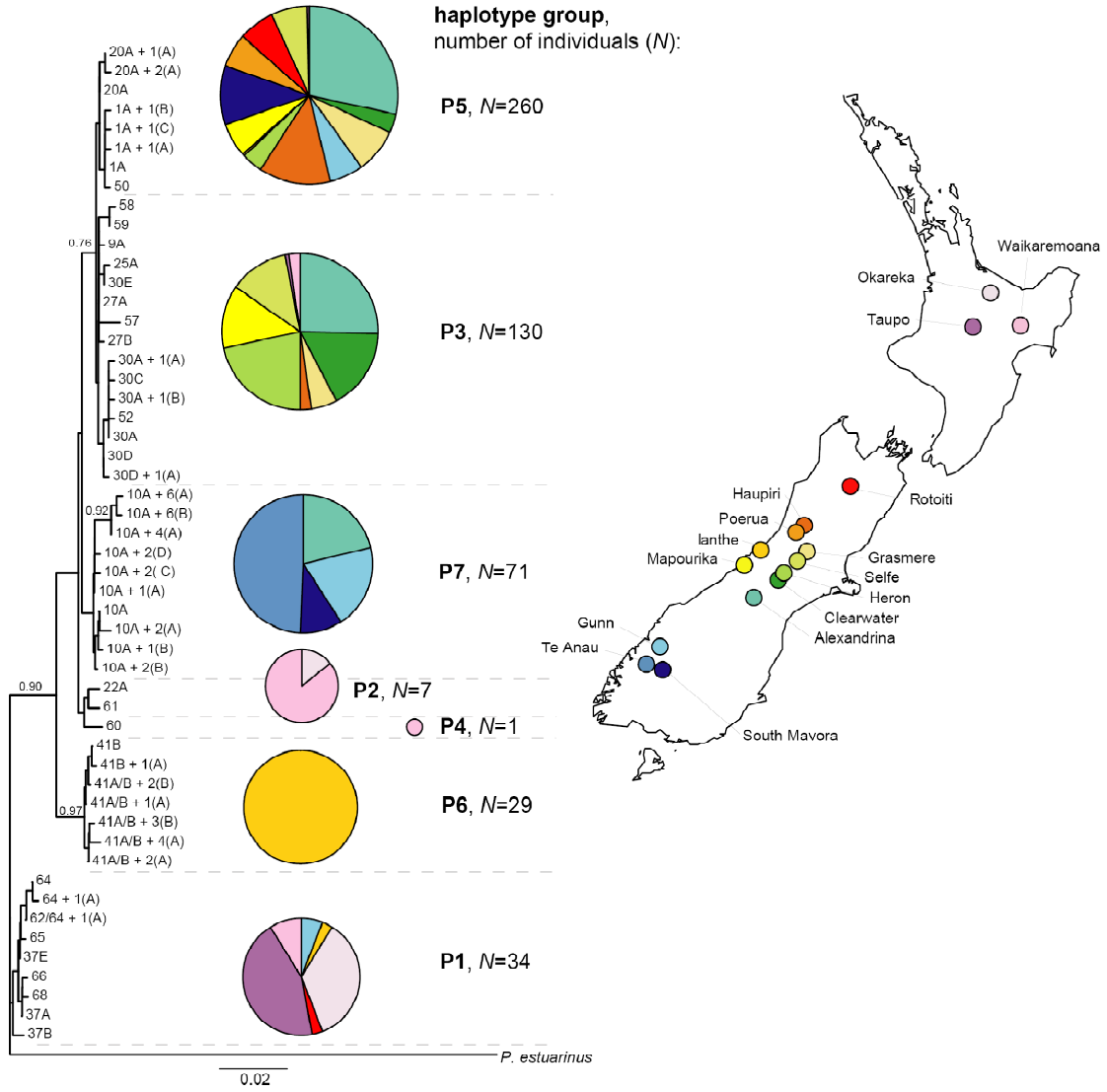


Fig. 2. Neighbour-joining tree representing 52 unique cytochrome-b haplotypes. Values to the left of nodes represent bootstrap support; only values > 70% are reported. The numbers at the ends of branches represent haplotype ID numbers. Pie charts represent the proportion of individuals from each lake (color-coded in the New Zealand map) found in each of 7 haplotype groups (P1-P7, separated with dashed lines). The haplotype groups were used as priors in DAPC analysis and, with the exception of P3 and P5, were separated by at least 5 nucleotide changes. The sizes of pie charts are proportional to log sample size in each of the groups. We calculated pie chart area as $A=\log_{10}(N^2\times\pi)$, where N is a number of individuals, and then scaled the sizes of pie charts as percentages of the *A* value relative to the largest group.

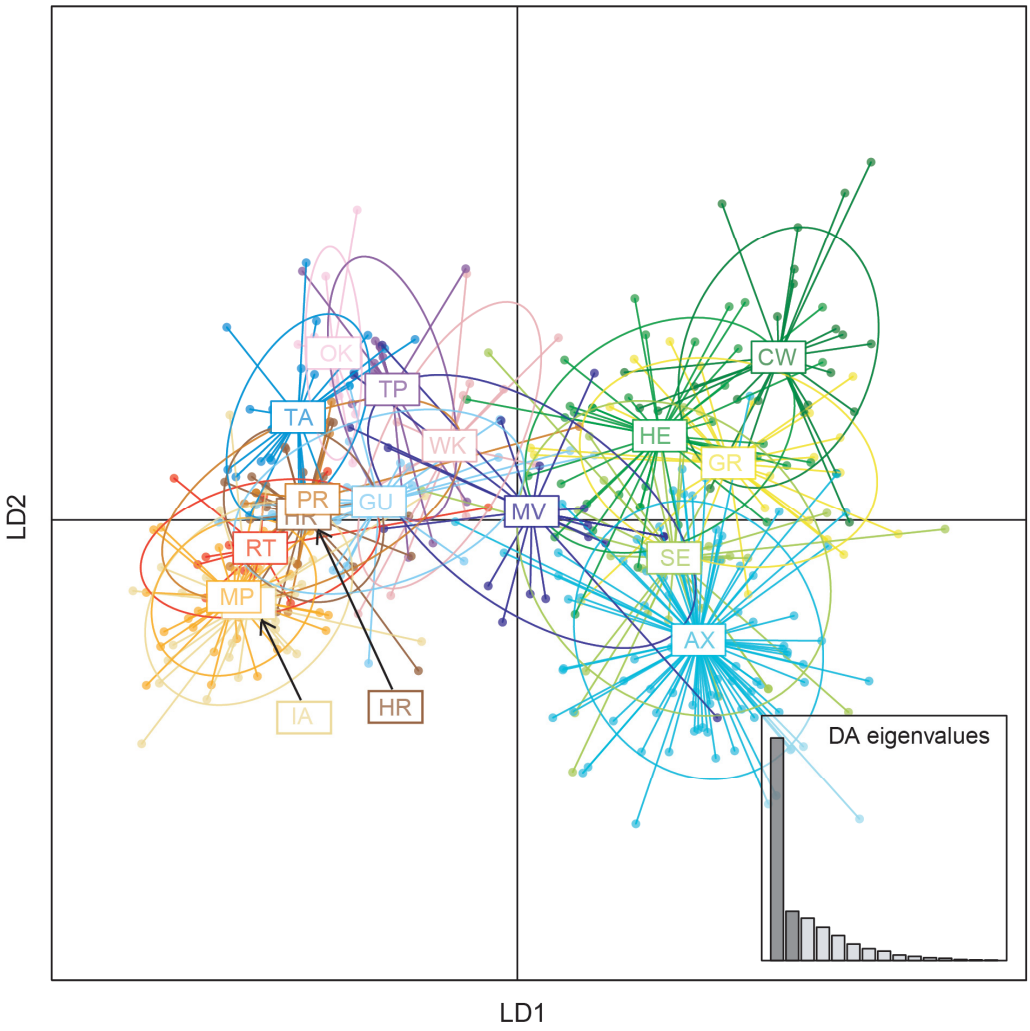


Fig. 3. Scatterplot of the first two axes of DAPC analysis using nuclear SNP data with population of origin as prior. Coloured circles represent individuals, and the prior groups are represented as inertia ellipses. Labels indicate prior population membership.

*Tests for a) association of the common 1A haplotype with asexuality, and b) a mitochondrial selective sweep*

We first compared the ability of a logit model with main effects of lake of origin and reproductive mode and a full model including both main effects and the effect of the interaction term to explain the distribution of the 1A haplotype within and across lakes, and

found no significant differences between the models ($\chi^2$=9.81, *P*=0.279, df=8). Thus, we used reduced models to evaluate whether there were significant main effects of lake of origin and/or reproductive mode (sexual vs. asexual) on the frequency of *P. antipodarum* bearing haplotype 1A. We found that both lake ($\chi^2$=16.90, *P*=0.031, df=8) and especially reproductive mode ($\chi^2$=204.66, $P=2\times10^{-46}$, df=1) influenced 1A frequency, with 1A much more likely to be found in asexual individuals.

The results of our regression analysis comparing across-population haplotype 1A frequency and nuclear genetic diversity showed that increased frequency of haplotype 1A was not associated with reduced nuclear genetic diversity ($R^2$=0.0002, $F_{(1,14)}$=0.003, *P*=0.958). This result suggests that genetic drift, which is expected to reduce diversity in both mitochondrial and nuclear genomes, is an unlikely cause of the commonness of haplotype 1A in asexuals, and suggests instead that selection on the mitochondrial genome and/or other cytoplasmic elements might be driving this pattern.

**Discussion**

Our nuclear SNP data revealed that asexual *P. antipodarum* harbor high genetic diversity and are often – but not always - closely related to coexisting sexual *P. antipodarum*. We also found that population genetic structure is strongly associated with lake of origin for both sexual and asexual *P. antipodarum*. These results are in part consistent with an earlier allozyme-based study indicating that asexual *P. antipodarum* are generally the product of multiple recent independent transitions to asexuality from sympatric sexual *P. antipodarum* (Dybdahl & Lively 1995), but depart from the findings of this previous study in revealing evidence for allopatric and/or non-recent origins for at least some asexuals. Consistent with this possibility, we also detected considerably more population structure for sexual than asexual *P. antipodarum* and found that asexual *P. antipodarum* were more likely than sexuals to harbor nuclear genotypes that did not group with other individuals from their lake of origin in a clustering analysis.

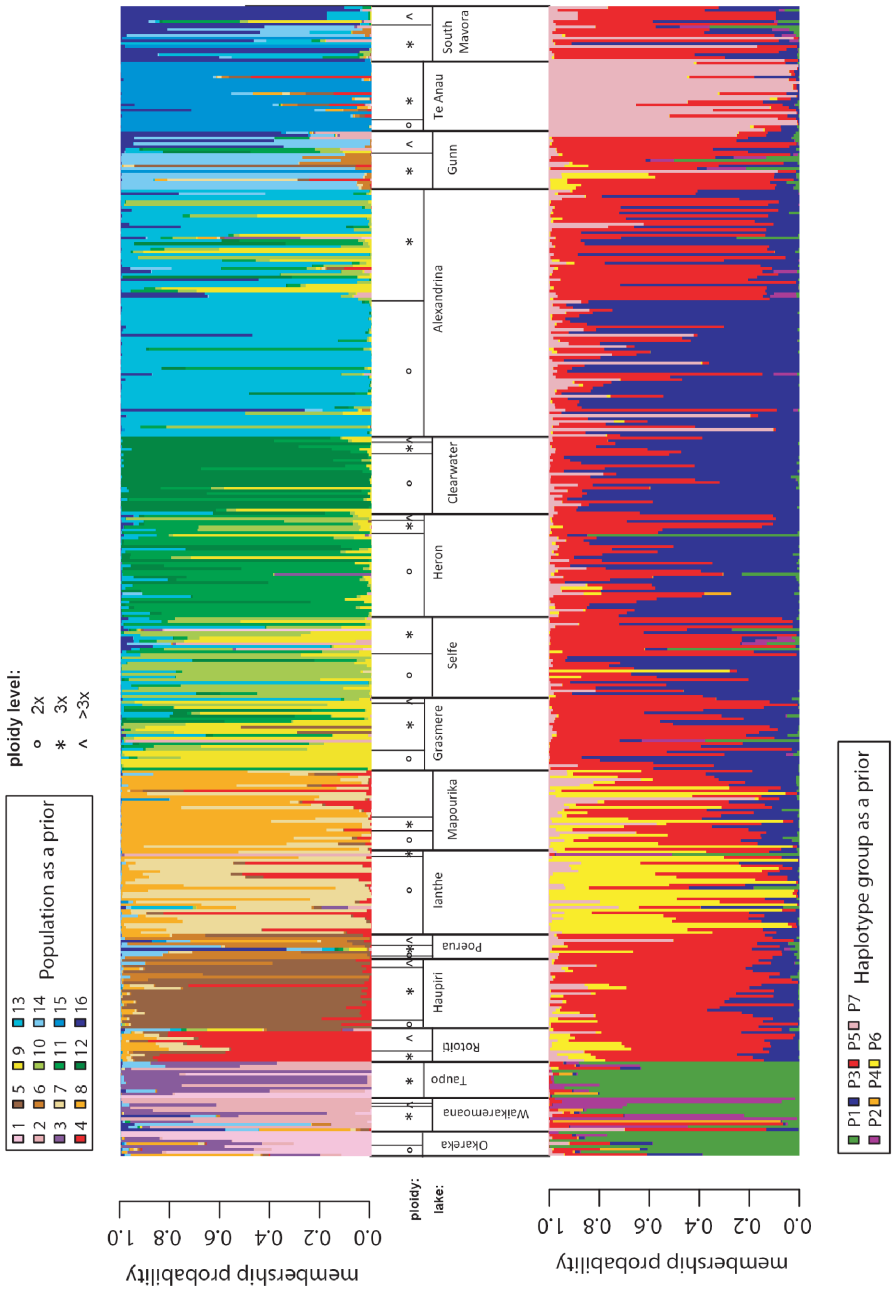


Fig. 4. Membership probabilities in clusters identified by DAPC analysis of nuclear SNP data using populations of origin (top) and mitochondrial haplotype group (bottom) as priors. Bars represent inferred membership probabilities in the prior groups (16 populations or 7 haplotype groups) for each individual.

We also found that a very common, geographically widespread, and recently derived cytochrome-b haplotype (1A) was strongly associated with asexuality (also see Neiman & Lively 2004; Neiman *et al.* 2011). Counter to predictions that the mitochondrial and nuclear genomes should show congruence within asexuals, asexual individuals harboring haplotype 1A often (but not always) had nuclear genotypes that were similar to those found in coexisting sexuals, indicating strong mitonuclear discordance within asexuals. Finally, higher relative frequency of this haplotype was not associated with reduced nuclear genetic diversity, suggesting that genetic drift alone is an unlikely cause of the commonness of 1A and pointing instead to a role for selection. Altogether, these data indicate that there is widespread mitonuclear discordance in asexual New Zealand *P. antipodarum*, and hint that at least some of this discordance may be linked to the spread of the cytoplasm containing haplotype 1A in asexuals.

Previous mtDNA-based studies of the phylogeography of *P. antipodarum* indicate that major genetic differences occur on a north/south axis (Neiman & Lively 2004; Neiman *et al.* 2011). This pattern was also evident in our mitochondrial data (Fig. 2). In contrast, the main phylogeographic pattern revealed by our nuclear SNP data was genetic differentiation between the east part of the south island and other regions, though a north-south division was still apparent. Altogether, these patterns are consistent with the major phylogeographic barriers described in other New Zealand taxa (Wardle 1963; McGlone 1985; Heads 1998; Trewick *et al.* 2000; Trewick 2001): the division between North and South Islands and between the eastern and western drainages of the Southern Alps on the South Island.

*Structure of asexual* P. antipodarum *populations*

The evolutionary consequences of migration are expected to differ for sexual and asexual populations because only the sexuals should experience introgression of migrant alleles. Migrant asexual lineages should instead contribute whole genotypes to the recipient population, which might be expected to leave a stronger signal in population genetic structure analyses than introgression of individual migrant alleles. Such a difference in the detectability of sexual vs. asexual migrants could help to explain why the proportion of asexual polyploid

*P. antipodarum* correctly assigned to their lake of origin by the clustering analysis was significantly lower than for diploid sexuals.

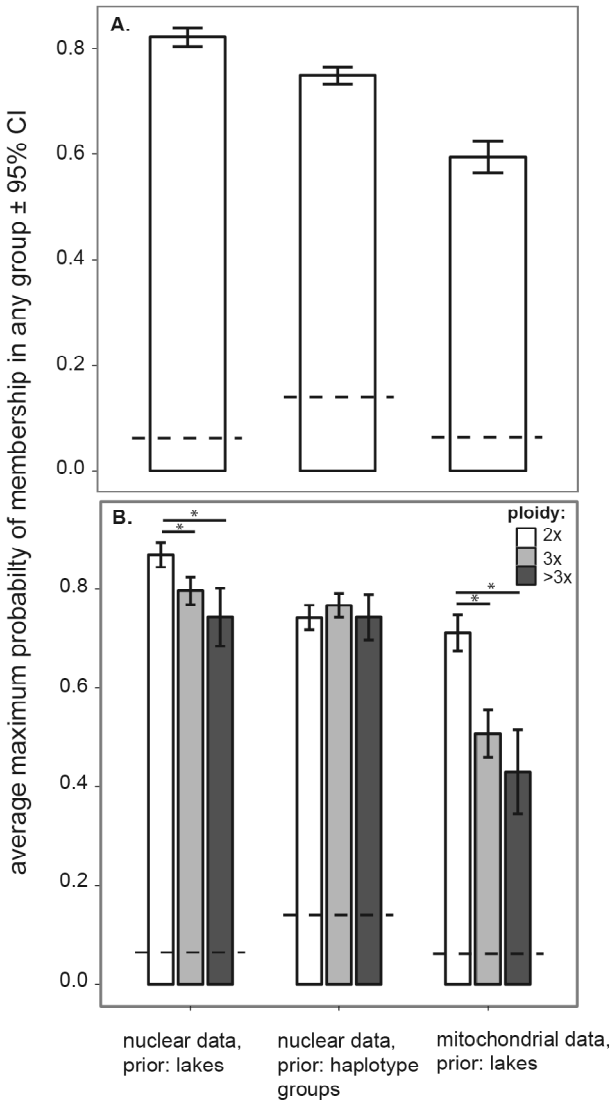


Fig. 5. Mean maximum probability of individual membership in any cluster assigned by DAPC analysis of nuclear SNP and mitochondrial haplotype data with population of origin or mitochondrial haplotype group as priors. A. Means for all unique genotypes. B. Means for all unique genotypes grouped by ploidy level. Error bars denote 95% confidence intervals. Dashed lines denote the null hypothesis for each of the analysis: with no structure in the data that can be explained by the priors each individual has 1/N probability of being placed in any cluster, where N is a number of prior clusters. $N_p$=16 populations, $N_h$=7 haplotype groups. Significant differences based on 95% confidence intervals are marked with an asterisk (*).

Regardless of potential detectability differences, our data suggest that at least some asexual *P. antipodarum* possess nuclear genotypes and/or mitochondrial haplotypes that are not of recent origin from coexisting sexuals. One possible explanation for this result is occasional ecological perturbations of sexual populations, which then recover to new genetic equilibria. It is plausible that it would take some time for asexual populations to reflect the new equilibrium allele frequencies of the coexisting sexual population, which could itself complicate cluster assignment of older asexual lineages representing pre-perturbation genetic equilibria. By this logic, we would predict more divergence between sexual and asexual lineages in environments where ecological perturbations are more likely. Because this hypothesis also posits that asexual individuals with genotypes that do not cluster with other individuals from their source populations represent relictual asexual lineages derived from relatively ancient (and perhaps extinct) sexual populations, we would also predict that these asexuals should show significantly more genetic divergence from any sexual *P. antipodarum* than asexual *P. antipodarum* that do cluster with other individuals from their lake of origin.

A different but non-mutually exclusive potential explanation for the existence of many asexual *P. antipodarum* with mitochondrial haplotypes and nuclear genotypes that do not readily group with other members of their source populations is that some of the asexual lineages are migrants derived from allopatric sexual populations. In contrast to the "perturbation" hypothesis detailed above, this "migrant" hypothesis predicts no consistent differences in genetic divergence from extant sexuals between "migrant" and "non-migrant" asexuals. The implications are that one could discriminate between the relative importance of these two hypotheses by using a coalescent-based analysis to compare the genetic divergence from sexual *P. antipodarum* of asexual *P. antipodarum* that cluster with their source population versus asexual *P. antipodarum* that do not. Rigorous execution of this analysis would require both sexual individuals sampled from many more populations than those included here as well as nuclear markers (e.g., microsatellites, sequence-based markers, or a large number of SNP markers) that permit more specific inference regarding ancestral states than our current set of SNPs.

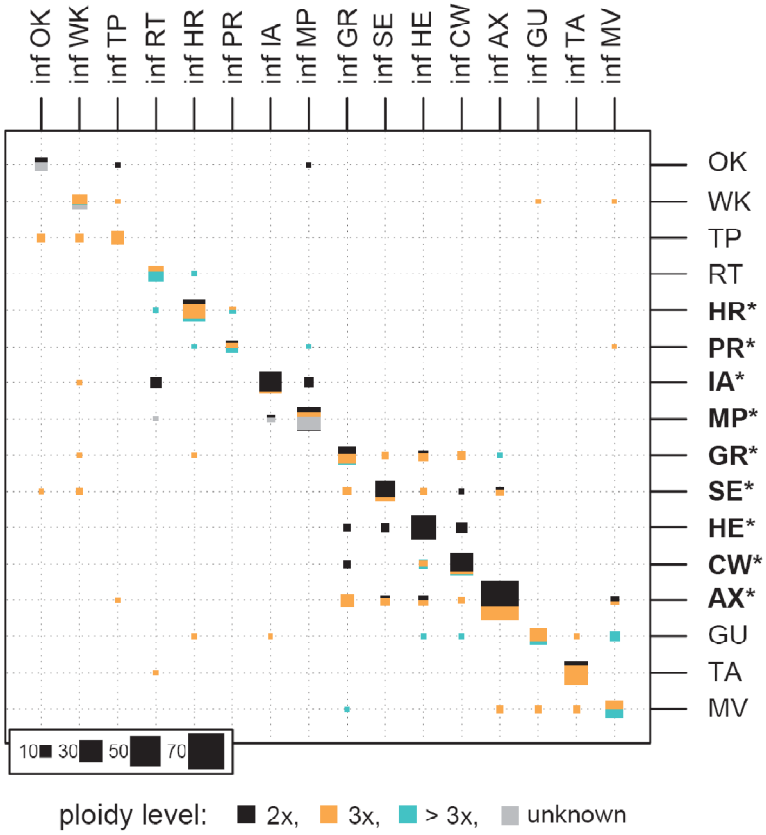


Fig. 6. Visual representation of the contingency table showing proportions of individuals assigned by DAPC analysis to the inferred posterior groups from their prior groups (population of origin). Rows represent prior groups and columns inferred (inf) groups. Population acronyms: OK: Okareka, WK: Waikaremoana, TP: Taupo, RT: Rotoiti, HR: Haupiri, PR: Poerua, IA: Ianthe, MP: Mapourika, GR: Grasmere, SE: Selfe, HE: Heron, CW: Clearwater, AX: Alexandrina, GU: Gunn, TA: Te Anau, MV: South Mavora. Populations that contain both sexual diploids and asexual polyploids and were thus used to test for the association of the correct reassignment with the reproductive mode (see Results for details) are marked with an asterisk (*).The sizes of squares are approximately proportional to numbers of individuals.

*Selection and mitochondrial haplotype 1A*

The commonness and the widespread occurrence of haplotype 1A and the fact that nuclear genetic diversity in the lakes where this haplotype is common was not reduced suggest that it may have spread by selection, as neutral processes such as genetic drift are expected to reduce

both mitochondrial and nuclear diversity. This haplotype was also found at disproportionately high relative frequency in asexual *P. antipodarum*. While we can only provide speculative suggestions regarding why a haplotype that may have spread via selection is so common in asexual *P. antipodarum*, potential explanations include but are not limited to scenarios where sexual *P. antipodarum* carrying 1A are more likely to give rise to new asexual lineages or where the asexual lineages carrying 1A have higher fitness than sexual *P. antipodarum* and/or other asexual lineages.

Our results highlight the importance of using data from both mitochondrial and nuclear genomes and from a large and geographically representative sample to characterize population genetic structure, enabling important new insights into the evolutionary history of sexual and asexual *P. antipodarum* that were not revealed by earlier mtDNA (Neiman & Lively 2004, Neiman *et al.* 2011) or allozyme-based studies (Dybdahl & Lively 1995). In our case, these data also illuminate the existence of intraspecific mitonuclear discordance in asexual lineages of *P. antipodarum*, which is surprising in light of the expectation that these genomes (unlike in sexuals) should be co-inherited. This result begs the question of the source of this variation, especially given the strong association between asexuality and a particular mitochondrial haplotype that our data suggest may have spread via selection. Evidence for widespread mitonuclear discordance in asexual organisms also sets the stage for the possibility of occasional violations of assumed strict asexuality (Schurko *et al.* 2009; also see Neiman *et al.* 2011).

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**Author contributions**

DP, JJ and MN designed research, analyzed data and wrote the paper. KL contributed to labwork and phylogenetic analyses. JJ and MN funded research.

**Literature Cited**

Altschul SF, Madden TL, Schaffer AA*, et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389-3402.

Avise JC (1994) *Molecular markers, natural history and evolution* Chapman & Hall, New York.

Burt A (2000) Perspective: Sex, recombination, and the efficacy of selection: was Weismann right? *Evolution* **54**, 337-351.

Case TJ, Taper ML (1986) On the coexistence and coevolution of asexual and sexual competitors. *Evolution* **40**, 366-387.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657-1659.

Dybdahl MF, Lively CM (1995) Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). *Journal of Evolutionary Biology* **8**, 385-398.

Dybdahl MF, Lively CM (1996) The geography of coevolution: comparative population structures for a snail and its trematode parasite. *Evolution* **50**, 2264-2275.

Fox JA, Dybdahl MF, Jokela J, Lively CM (1996) Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). *Evolution* **50**, 1541-1548.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* **34**, 397-423.

Gompert Z, Forister ML, Fordyce JA, Nice CC (2008) Widespread mito-nuclear discordance with evidence for introgressive hybridization and selective sweeps in *Lycaeides*. *Molecular Ecology* **17**, 5231-5244.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity* **86**, 485-486.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclear Acids Symposium Series* **41**, 95-98.

Heads M (1998) Biogeographic distribution along the Alpine Fault, New Zealand. *Biological Journal of the Linnean Society* **63**, 161-176.

Holton TA, Pisani D (2010) Deep genomic-scale analyses of the metazoa reject Coelomata: evidence from single- and multigene families analyzed under a supertree and supermatrix paradigm. *Genome Biology and Evolution* **2**, 310-324.

Jokela J, Dybdahl MF, Lively CM (2009) The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *American Naturalist* **174**, S43-S53.

Jokela J, Lively CM, Dybdahl MF, Fox JA (1997) Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. *Ecology* **78**, 452-460.

Jokela J, Mutikainen P (1995) Effect of size-dependent muskrat (*Ondatra zibethica*) predation on the spatial distribution of a freshwater clam, *Anodonta piscinalis* Nilsson (Unionidae, Bivalvia). *Canadian Journal of Zoology* **73**, 1085-1094.

Jombart T (2008) *adegenet*: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**, 15.

Kearney M (2005) Hybridization, glaciation and geographical parthenogenesis. *Trends in Ecology & Evolution* **20**, 495-502.

Lively CM (1987) Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature* **328**, 519-521.

Maynard Smith J (1978) *The evolution of sex* Cambridge Univeristy Press, Cambridge.

McGlone MS (1985) Plant biogeography and the late Cenozoic history of New Zealand. *New Zealand Journal of Botany* **23**, 723-749.

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* **4**, 792-794.

Murtaugh PA (1988) Use of logistic regression in modelling prey selection by *Neomysis mercedis*. *Ecological Modelling* **43**, 225-233.

Neiman M, Larkin K, Thompson AR, Wilton P (2012) Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snail. *Heredity* **109**, 57-62.

Neiman M, Lively CM (2004) Pleistocene glaciation is implicated in the phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail. *Molecular Ecology* **13**, 3085-3098.

Neiman M, Meirmans S, Meirmans PG (2009) What can asexual lineage age tell us about the maintenance of sex? *Annals of the New York Academy of Sciences* **1168**, 185-200.

Neiman M, Paczesniak D, Soper DM, Baldwin AT, Hehman G (2011) Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution* **65**, 3202-3216.

Neiman M, Schwander T (2011) Using parthenogenetic lineages to identify advantages of sex. *Evolutionary Biology* **38**, 115-123.

Norusis MJ (1990) *SPSS advanced statistics user's guide.* SPSS Inc., Chicago.

R Development Core Team (2009) R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Schurko AM, Neiman M, Logsdon JM (2009) Signs of sex: what we know and how we know it. *Trends in Ecology & Evolution* **24**, 208-217.

Tamura K, Peterson D, Peterson N*, et al.* (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731-2739.

Toews DPL, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* **21**, 3907-3930.

Tomiuk J, Guldbrandtsen B, Loeschcke V (2009) Genetic similarity of polyploids: a new version of the computer program POPDIST (version 1.2.0) considers intraspecific genetic differentiation. *Molecular Ecology Resources* **9**, 1364-1368.

Trewick SA, and G. P. Wallis (2001) Bridging the "beech-gap": New Zealand invertebrate phylogeography implicates Pleiostocene glaciation and Pliocene isolation. *Evolution* **55**, 2170-2180.

Trewick SA, Wallis GP, Morgan-Richards M (2000) Phylogeographic pattern correlates with Pliocene mountain building in the alpine scree weta (Orthoptera, Anostostomatidae). *Molecular Ecology* **9**, 657-666.

Wallace C (1992) Parthenogenesis, sex and chromosomes in *Potamopyrgus*. *Journal of Molluscan Studies* **58**, 93-107.

Wardle P (1963) Evolution and distribution of the New Zealand flora as affected by Quaternary climate. *New Zealand Journal of Botany* **1**, 3-17.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Wilton PR, Sloan DB, Logsdon Jr JM, Doddapaneni H, Neiman M (2013) Characterization of transcriptomes from sexual and asexual lineages of a New Zealand snail (*Potamopyrgus antipodarum*). *Molecular Ecology Resources* **13**, 289-294.

# Chapter III

## Faster clonal turnover in high-infection habitats provides evidence for parasite-mediated selection

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**Abstract**

According to the parasite hypothesis for sex, parasite-mediated selection against common clones counterbalances the reproductive advantage of asexual lineages, which would otherwise outcompete sexual conspecifics. Such selection on the clonal population should lead to faster clonal turnover in habitats where selection by parasites is stronger. We tested this prediction by comparing the genetic structure of clonal and sexual populations of freshwater snail *Potamopyrgus antipodarum* between two time points over a 4-year period in three depth-specific habitats in Lake Alexandrina (South Island, New Zealand). These habitats differ in both risk of infection by castrating trematodes and the proportion of sexual individuals. We found that between 2003 and 2007 the clonal structure changed significantly in shallow and mid-water habitats where prevalence of infection is high, but not in the deep habitat, where prevalence is low. In contrast, the genetic structure (allele frequencies, F-statistics) of the co-existing sexual population did not change; therefore the change in clonal structure cannot be related to genetic changes in the sexual population. Taken together, our results show more rapid clonal turnover in high infection habitats, which gives support for the parasite hypothesis for sex.

## Introduction

Clonal diversity of asexual populations is an outcome of two opposing processes: the emergence of new clones and the extinction of existing lineages. Usually, new clones are derived spontaneously from within sexual lineages, through hybridization between sexual lineages, or by mutational diversification of existing clonal lineages (Simon *et al.*, 2003). However, the rate of emergence of new clones from sexual populations, and the extent to which it contributes to clonal diversity, is not clear. For example, Pongratz *et al.* (1998) found that parthenogenetic planarians from populations that are sympatric with their sexual relatives harbour twice as much genetic diversity as isolated parthenogenetic populations. In contrast, King *et al.* (2011a) found no correlation between the local frequency of sexual individuals and the level of clonal diversity in mixed (sexual and asexual) populations of *Potamopyrgus antipodarum* snails in streams.

One of the fundamental differences between sexual and asexual populations is that, in sexual populations natural selection sorts genes, while in asexual populations it sorts genotypes (Haldane, 1924; Fisher, 1930). While recombination allows rapid sweeps of advantageous alleles in response to selection in sexuals, natural selection is predicted to reduce the genotypic diversity of clonal populations. Selection on the clonal population is expected to reduce genetic diversity and evenness of the population, as it operates on the variance in fitness among clones. The differences in fitness among clones are expected to effectively sort high-fitness clones from low-fitness clones (Spitze, 1991; Scheiner & Yampolsky, 1998; Vanoverbeke & De Meester, 2010). Thus clonal diversity is reduced by the extinction of clonal lineages due to natural selection and stochastic processes.

While directional selection and drift may lead to a rapid reduction in genetic diversity, negative frequency-dependent selection is predicted to promote clonal diversity. Parasite-mediated, frequency-dependent selection is one of the major hypotheses for the maintenance of sexual reproduction (Red Queen hypothesis, Haldane, 1949; Jaenike, 1978; Hamilton, 1980). Theoretical models of host-parasite coevolution have demonstrated that frequency-dependent selection may also account for maintenance of clonal diversity (Lively & Howard, 1994) and empirical support for this phenomenon now exists. For example, in a

study by Wolinska & Spaak (2009), populations of lake *Daphnia* that were subject to infection by virulent parasites had a higher evenness and clonal diversity than populations without infections. King *et al.* (2011a) found that clonal diversity in stream populations of *P. antipodarum* was positively and significantly correlated with infection frequency. Nonetheless, the role that frequency-dependent selection plays, in general, remains unclear, as we lack comprehensive data on temporal dynamics of population genetic parameters in clonal populations.

In this study, our aim was to evaluate the relative importance of processes that affect clonal diversity and population genetic structure of coexisting asexual and sexual lineages in a well-studied lake population. In a previous study, Fox *et al.* (1996) described the clonal diversity and distribution of sexual and asexual snails in three habitat zones of Lake Alexandrina in 1994. Their main findings were that: (1) the proportion of asexual individuals increased with depth, (2) clonal diversity was very high in all habitats, and (3) that each habitat contained a distinct assemblage of clones. This study, however, represents only a snapshot in time, and does not consider the possibility of temporal dynamics that may exist in the different habitats. Here we examined the temporal and spatial dynamics of the genetic structure in the same population in years 2003 and 2007, i.e., 9 and 13 years after the Fox *et al.* (1996) study.
Assuming that the rate of origin of new clones is an important factor contributing to clonal diversity, we predicted that clonal diversity should have remained high or increased in habitats where sexuals are more common, because new clones originate from the sexual population in this snail (Dybdahl & Lively, 1995b). We also predicted that the rate of emergence of new clones would be lower in the deep habitats where sexuals are rare, and therefore clonal diversity should be lower in the deep habitat. To test these predictions, we describe the temporal and spatial dynamics of the clonal population in reference to the genetic structure of the coexisting sexual population.

Earlier studies on the Lake Alexandrina population have shown strong parasite pressure in the shallow, but not in the deep habitats of the lake (Jokela & Lively, 1995a; Jokela & Lively, 1995b). The parasite prevalence of castrating trematodes found in the present study was also consistent with this pattern. Parasite pressure has been suggested to drive the temporal

dynamics of the common clones in shallow and mid-water habitats (Jokela *et al.*, 2009). Assuming that frequency-dependent selection is relatively weak in the deep, we predicted that the temporal dynamics in the deep are not as pronounced as in the shallow and mid-water habitats. Earlier studies have not evaluated temporal clonal dynamics in the deep habitats. Alternatively, clones that become common could be resistant migrants. Lake-specific local adaptation by the parasites has been shown to be very strong in this system (Lively *et al.*, 2004); hence, migrants could carry resistance genotypes that are difficult to match by the local parasite population (Altermatt *et al.*, 2007). To test this hypothesis, we calculated genetic similarity between sexual genotypes, rare clones and common clones. Knowing that the sexual populations of *P. antipodarum* are quite divergent between lakes (Wright's Fst=0.17, Dybdahl & Lively, 1996), our prediction was that, if common clones are migrants, they should be more dissimilar to sexual genotypes than rare clones.

Our study is the first detailed study on temporal dynamics of clonal populations in habitats that differ according to parasite pressure. As predicted by the parasite hypothesis for sex, we found that the clonal structure in the deep habitat did not change as rapidly as in the shallow and mid-water habitats, and that the high frequency of sexual snails in the shallow habitat had no effect on the clonal diversity or evenness of the coexisting asexual population. Our results highlight the importance of considering ecological interactions as a potential driver of short-term evolutionary responses in clonal assemblages.

## Materials and methods

### Study system

*Potamopyrgus antipodarum* is a Prosobranch snail, native to New Zealand freshwater habitats. Populations of the snail are often mixtures of diploid, obligately sexual individuals with triploid, obligately asexual individuals in variable proportions (Winterbourn, 1970; Lively, 1987; Wallace, 1992; Dybdahl & Lively, 1995b). Apomictic clones have a high clonal diversity and are ecologically similar and genetically related to their co-existing sexual counterparts (Dybdahl & Lively, 1995b; Jokela *et al.*, 1997b; Jokela *et al.*, 1997a).

Lake Alexandrina is a mesotrophic sub-alpine lake (Ward & Talbot, 1984) on the South Island of New Zealand. Sexual and asexual snails coexist in this lake, and their relative proportions vary according to depth (Fox *et al.*, 1996). The proportion of asexuals is higher in the deep-water habitat, while the proportion of sexual individuals is higher in the shallow areas (Fox *et al.*, 1996), where also the infection risk by digenetic trematodes is also the highest (Jokela & Lively, 1995a; Jokela & Lively, 1995b).

**Sample and infection data collection**

Snails were collected from five sites in Lake Alexandrina (New Zealand, South Island) (Fig.1; GPS coordinates of sampling sites are in Supporting Information, Table S1) between mid-January and mid-February 2003 and 2007. At each site, three depth-related and easy to distinguish habitats were sampled: (i) shallow (<0.5 m, near the shore bank, defined by willow roots), (ii) mid-water (1-3 m, dominated by the macrophyte *Isoetes alpinus*) and (iii) deep (4-6 m, dominated by the macrophytes *Elodea canadensis*, *Myriophyllum triphyllum* and *Chara* sp.) (Ward & Talbot, 1984). Snails were collected by pushing a kicknet through vegetation. Collections were done by the same persons (JJ and CML) each year. Snorkelling equipment was used to sample at greater depths. Samples were transported to Edward Percival Field Station (Kaikoura, New Zealand), where they were divided in two parts. One part was snap frozen and stored in -80˚C until genotyping. The remaining snails were kept alive in plastic containers, with water changed every other day, and fed *Spirulina* algae at libitum until dissection. Within two weeks after collection 89 to 202 snails per sample were examined for infection under a dissecting microscope. We recorded snail gender and, for infected snails, we determined the parasite species. The genetic and infection data are therefore derived from the same random sample, but not the same individuals: for each individual snail we obtained either infection or genotype data.

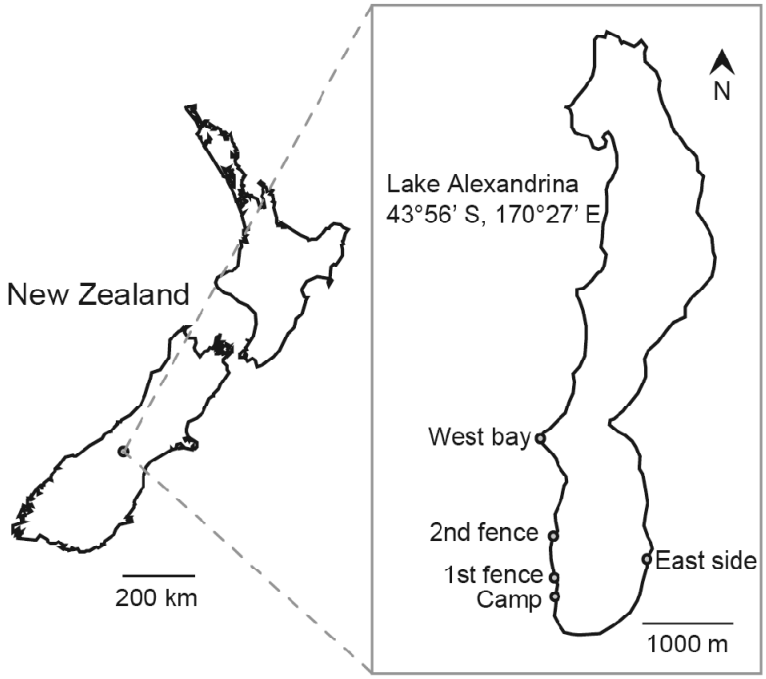


Figure 1. Map of New Zeland, showing the location of Lake Alexandrina and sample-site localities across the lake.

**Genotyping**

Samples were genotyped using 9 allozyme loci (6PGD, PEP-D, MPI, IDH1, IDH2, AAT1, AAT2, PGM1, PGM2) using cellulose acetate electrophoresis. The genotyping method is described in detail in Fox *et al.* (1996) and Jokela *et al.* (2009). For identification of the clonal genotypes, we followed the same method as described in Jokela *et al.* (2009): diploid individuals were identified by the presence of two alleles at at least one locus, and diploids were distinguished from triploid heterozygotes by the presence of asymmetric banding patterns in the latter. Individuals homozygous at all loci for which ploidy level could not be assigned based on banding pattern were checked for presence of common genotypes with program GENCLONE version 2.0 (Arnaud-Haond & Belkhir, 2007), which estimates the probability ($P_{sex}$) that a given genotype is a product of a distinct reproductive event . For none of the homozygous multilocus genotypes $P_{sex}$ values were below 0.05, suggesting that they were most likely products of sexual reproduction, and were therefore included in the sexual group.

Individuals for which the allozyme banding pattern was not readable at all loci were only assigned a genotype number when unambiguous assignment was possible. Sample sizes and proportions of sexual, asexual, and unresolved individuals are shown in the Supporting Information (Table S2). Higher proportion of individuals for which the clonal identity could not be assigned for samples from 2003 result from a freezer failure in 2004, which increased the number of individuals for which the enzymes were degraded to a point where we could not reliably genotype them. It is however unlikely that the unresolved samples represent other than random set of genotypes and would therefore bias the results.

**Genetic analysis**

*Sexual population*

We calculated the proportions of sexual (diploid and homozygote) and asexual (triploid) snails in each sample (Table S2) of genotyped snails. We then used a generalized linear model to test how the frequency of the snails with the two different reproductive modes (sexual, asexual) varied among years (2003, 2007), habitats (shallow, mid-water) and sites (5 sites). We coded the response variable as binomial and used a logit link function in the model. Only samples from shallow and mid-water habitats were included in the analysis, as in the deep there was only one homozygote individual in 2003 and no sexual (either diploid or homozygote) individuals in 2007. We used Wald $\chi^2$ statistics to test the main effects and all interaction terms. The model was fitted using IBM® SPSS Statistics version 19.

We then tested if the genotype frequencies of sexuals were in agreement with Hardy-Weinberg equilibrium using exact tests. *P*-values were obtained with Markov chain method as implemented in TFPGA version 1.3. (Miller, 1997). As one locus (PEP-D) showed significant deviations from Hardy-Weinberg equilibrium, we excluded this locus from subsequent F-statistic calculations, but included it when we assigned individuals to different ploidy groups (see Results).

To test for possible differentiation of the sexual population at spatial (among sites or habitats) and temporal (between years) scales, we calculated pairwise $F_{st}$ between samples. We also tested for significance of the calculated $F_{st}$ values with permutation test available in Fstat version 2.9.3 (Goudet, 1995). None of the pairs of loci showed significant genotypic linkage disequilibrium (after correcting for multiple tests at 5% risk level; results not shown).

*Clonal structure*

For all analyses described below the frequency of clone was calculated as the frequency among all triploid individuals for which we could unambiguously assign clonal identity. We used a Detrended Correspondence Analysis (DCA) (Hill & Gauch, 1980) based on frequencies of clones to visualize samples according to their clonal structure using the R-package 'Vegan' (R Development Core Team, 2009; Oksanen *et al.*, 2010) We then fit structuring factor variables (site, year, habitat) as well as the continuous variables (prevalences of the four most common parasite species: *Microphallus livelyei*, *Notocotylus gippyensis Telogaster opisthorchis* and *Apatemon* sp., total trematode parasite prevalence, proportion of males) into the DCA using a function 'envfit'. This function finds vector or factor averages for provided external variables and uses permutation tests to assess significance. We used the function 'ordiellipse' to draw dispersion ellipses based on significant structuring factors (habitat and year) using standard deviation of point scores with a confidence limit of 0.90 (Figure 4). For DCA analysis we used default settings which included down weighting the importance of clones with frequency less than 0.2, as otherwise these clones would have had disproportionately high influence on the ordination axes (Oksanen *et al.*, 2010).

For clonal richness, evenness and heterogeneity analyses, we calculated the relative frequency of each clone for all habitats and years, pooling across sites to achieve sufficient sample size (Figure 3). We decided to pool samples from different sites because the above DCA analysis and an earlier study by Fox *et al.* (1996) found that the among-site variation in clonal structure was lower than the structure by habitats. Re-analysis of the partial chi-square tests examining the relative contribution of habitat and sampling site to spatial structure of 10 most common clones (Table 3 in Fox *et al.* 1996) indicated that the effect of transect was on average statistically not significant (average $P$=0.13) and the effect of habitat was 15-fold higher than the transect, being highly statistically significant for the 10 most common clones (average $P$=0.009).

Clonal richness was measured using the Stoddart index $G_0$ (Stoddart & Taylor, 1988). $G_0$ is a measure of genotypic richness ranging from 1 to $N$ (where $N$ is a sample size); therefore to correct for variation in sample size between habitats we randomly selected (with replacement)

a subsample of 40 individuals from each group. After 1000 bootstraps we recorded mean and standard deviation (Figure 2).

Evenness of the frequency distribution of the clones was measured with the index $E_5$ (Ludwig & Reynolds, 1988). $E_5$ index values range from 0 to 1, where low values indicate unequal proportions of clones in the sample and high values indicate more equal proportions of different clones. We calculated mean and standard deviation from 1000 bootstrap replicates (Figure 2).

Clonal heterogeneity, which simultaneously characterizes richness and evenness, was described using Simpson's complement D (Simpson, 1949; Arnaud-Haond *et al.*, 2007). It describes the probability of encountering different clones when randomly drawing two individuals from the sample. Mean and standard deviation were calculated from 1000 bootstrap replicates (Figure 2).

We used Matlab 7.9.0 (MathWorks, Natick, MA) for calculation of all indices and for bootstrapping.

*Genetic identity*

We calculated genetic similarity between different multilocus genotypes using the band-sharing measure as implemented in POPDIST v.1.2.1 (Tomiuk *et al.*, 2009). This genetic identity measure is one of a few that can be used to compare individuals of different ploidy levels. We aimed to compare genetic similarities between sexuals, common clones, and rare clones. Again, samples from different sites for given habitat and year combinations were pooled. A clone was defined as "common" if its frequency was above 1.5% in any of the samples, and the remaining clones were treated as "rare". When using the cut-off frequency of 1.5% the common clones represent 52% of the clonal population with 41 unique genotypes. We calculated pairwise identity between all genotypes within these three groups: (i) sexual multilocus genotypes (number of unique genotypes: $N$=296), (ii) rare clones ($N$=404) and (iii) common clones ($N$=41). We then calculated kernel density of these distributions using default settings for the "density" function in R (R Development Core Team, 2009) .

We used permutation tests in order to test if the mean identity values within and/or between rare clones, common clones and sexuals were significantly higher or lower than expected by chance. Previous studies (Fox *et al.*, 1996) showed that that there are differences in genetic

structure of asexuals between habitats. Our own results confirmed these findings and moreover showed that genetic structure of asexuals also changes in time (see Results and Figure 4). Therefore as units of comparison we used samples from a given habitat in a given year. The number of common clones varied between samples (Table 2), and in the shallow habitat there were too few common clones (four in 2003, six in 2007) to perform meaningful comparisons; so only samples from mid-water and deep habitats were used in subsequent analysis. In order to control for variable sample size, we randomly sampled a number of rare clones and a number of sexual genotypes equal to the number of common clones (Table 2, *N*). Rare clones were sampled from the pool of rare clones in the given habitat and year, and sexuals were sampled from the pool of sexual genotypes in the whole dataset. We decided to pool all sexuals, because the sexual population did not show signs of differentiation between sites, habitats or years (see Results for details). For each of the samples we performed three replicate analyses, by randomly sampling a set of rare clones and sexuals for comparisons. We calculated pairwise identity between multilocus genotypes using the band-sharing measure as implemented in POPDIST v.1.2.1 (Tomiuk *et al.*, 2009). Then, for each of the replicate comparisons, we calculated means of all the pairwise identities within and between the groups (common clones, rare clones and sexuals). To test for significance, the observed means were compared to 1000 mean identities obtained by permuting genotypes across groups and calculating within- and across- group mean identities for each permutation (Table 2). Permutations were performed using Matlab 7.9.0 (MathWorks, Natick, MA).

**Results**

We genotyped 66 individuals from each site/habitat/year combination. We were able to assign both ploidy level and, for triploid individuals, unambiguously assign them as a member of a clonal genotype for 73-100% of the individuals (Table S2). As expected based on earlier studies (Fox *et al.*, 1996), the proportion of sexuals (assessed from the genotyping data) in the shallow habitat was higher than in the mid-water habitat (Table 1, between habitats: Wald $\chi^2$=161.28, $P$<0.001, $df$=1). In addition, we found that the proportion of sexual individuals increased by one third in the shallow and by over three-fold in the mid-water habitat between years 2003 and 2007 (Table 1, year*habitat: Wald $\chi^2$ = 13.34, $P$ < 0.001, $df$=1). The proportion of sexual snails also varied between sites (Wald $\chi^2$ = 28.59, $P$ < 0.001, $df$ = 4), and

the interaction between sites and habitat was statistically significant (Wald $\chi^2 = 10.81$, $P = 0.029$, $df=4$). In contrast to earlier studies (Fox *et al.*, 1996), we found very few sexual individuals in the deep habitat (only one homozygous individual in 2003 and no diploids or homozygotes in 2007). Detailed sample size, proportion of sexual and asexual individuals and numbers of clones per site are given in the Supporting Information (Table S2). Allele frequencies at each locus for the sexual population are in the Supporting Information (Table S3). Observed genotype frequencies for the sexuals were largely in agreement with Hardy-Weinberg equilibrium (Supporting Information, Table S4).

Table 1. Percentage of sexual snails by habitat and year in samples from Lake Alexandrina. Values for shallow and mid-water habitats are estimated marginal means ± SE based on the generalized linear model described in the text. Samples from the deep water habitat could not be included in the model, because in 2007 there were no sexual snails. Only one presumably sexual (homozygous at all loci) snail was found in the deep habitat in 2003.

| habitat | year | |
|---|---|---|
| | 2003 | 2007 |
| shallow | 54±2.9 | 75±2.5 |
| mid-water | 10±1.8 | 45±2.8 |
| deep | 0.3* | 0 |

* arithmetic mean

The measure of population subdivision ($F_{st}$) was calculated between all pairs of samples (site, habitat and year combinations), and was tested for significance using permutation tests. The $F_{st}$ values were generally low (mean: -0.003; min-max: -0.092- 0.055), and only 21 out of 190 comparisons were significant at 95% confidence level. After correction for multiple tests only one of these tests remained significant. The matrix of pairwise $F_{st}$ values is presented in Supporting Information (Table S5).

Overall clonal richness was very high. We found 420 unique multilocus genotypes among 1208 triploid individuals for which a unique genotype could be unambiguously assigned (35 unique genotypes per 100 triploid individuals). Based on the bootstrapped confidence intervals, clonal richness was similar across all habitats, apart from shallow habitat in 2007 where it dropped to one fifth of its value in 2003 (Figure 2). Samples with the lowest richness (shallow 2007) also had the lowest evenness (Figure 2). The low richness and evenness values for shallow habitat in 2007 were driven by a single clone (number 47) being present at very high frequency (almost 60% of all triploid individuals) and only very few other clones

being present in the samples (Figure 2, Figure 3). The clonal heterogeneity index, which combines information on both richness and evenness, was highest for samples from the deep habitat. The sample from shallow habitat in 2007 had a clearly lower heterogeneity index than the other samples (Figure 2). Overall, there were no obvious differences among years in clonal diversity indexes, with the exception of shallow habitat samples between 2003 and 2007.

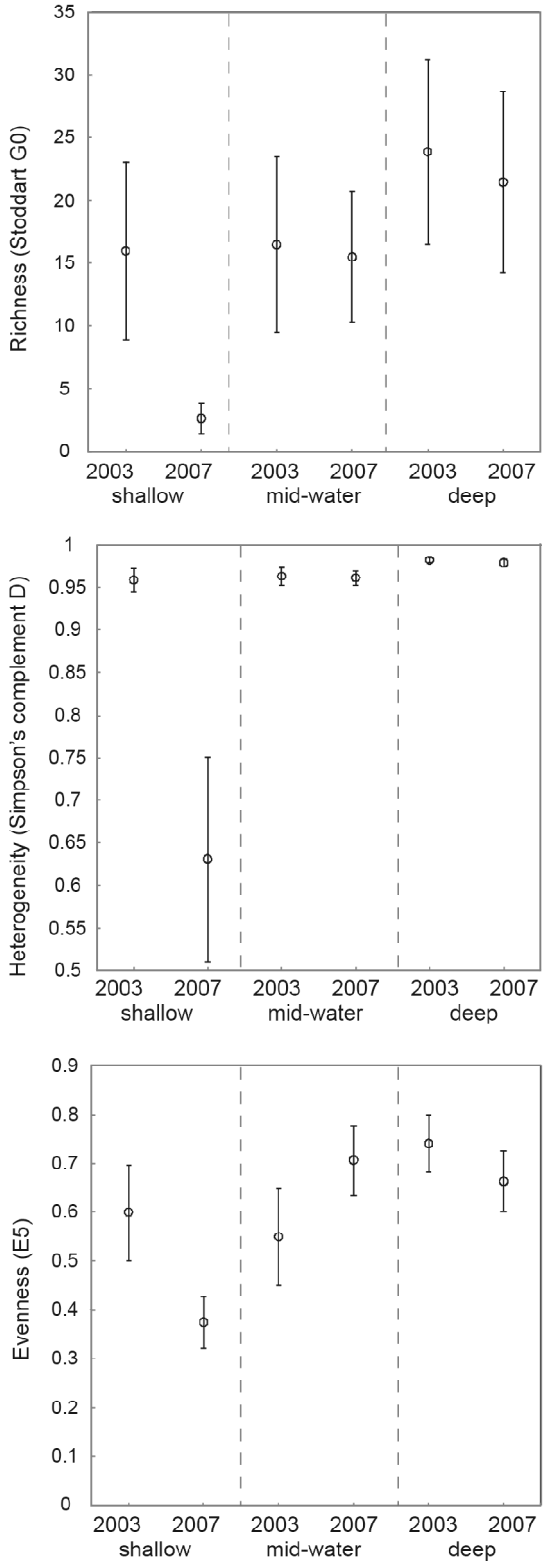


Figure 2. Mean and confidence interval (95%) of diversity indexes (clonal richness, heterogeneity and evenness). Confidence intervals were estimated from 1000 bootstrapped index values. Clonal richness index Stoddart G0 is calculated with correcting for sample size by sub-sampling units of 40 individuals.

Most of the common clones were different among habitats, and none of the clones with the highest frequency in one habitat were dominant in another (Figure 3), indicating that clonal population was structured by depth, corroborating the results an earlier study (Fox *et al.*, 1996).

We compared the clonal composition among samples with Detrended Correspondence Analysis. The first axis (DCA1) separated the samples by habitat, and the second axis (DCA2) separated the samples by year. Permutation tests showed a significant correlation between the DCA of clonal composition for year ($R^2$=0.384, $P$=0.003) and for habitat ($R^2$=0.8146, $P$=0.001), but there was no correlation with sampling site ($R^2$=0.068, $P$=0.358).
The 90% confidence ellipses discriminated the samples of different habitats from each other, with the exception of mid-water 2003, which had some overlap with the deep. The change in clonal structure between years, however, was limited to shallow and mid-water habitats, as the 90% confidence ellipses are overlapping for both years for samples from the deep habitat (Figure 4). Inclusion of the the continuous variables obtained from samples screened for parasites revealed a significant correlation between the DCA of clonal composition and the frequencies of total infection ($R^2$=0.4311, $P$=0.001), *Microphallus livelyei* ($R^2$= 0.4877, $P$= 0.001), *Notocotylus gippyensis* ($R^2$=0.5286, $P$= 0.001) and the frequency of males ($R^2$= 0.5505, $P$= 0.001). There was no significant correlation with the frequency of *Telogaster opisthorchis* ($R^2$= 0.1836, $P$= 0.061) or *Apatemon* sp. ($R^2$= 0.1765, $P$= 0.069) (Figure 4). All the significantly correlated variable vectors (namely, proportions of total infection, *Microphallus livelyei*, *Notocotylus gippyensis,* and the frequency of males) point in the same direction (along DCA1 axis) as the vector of the habitat variable, demonstrating the presence of an across-habitat cline in both infection frequency by these two most common parasite species and the frequency of sexual individuals (as shown in previous studies, e.g. Jokela & Lively, 1995b). Detailed information on parasite and male frequencies as well as sample sizes are presented in Supporting Information (Table S6).

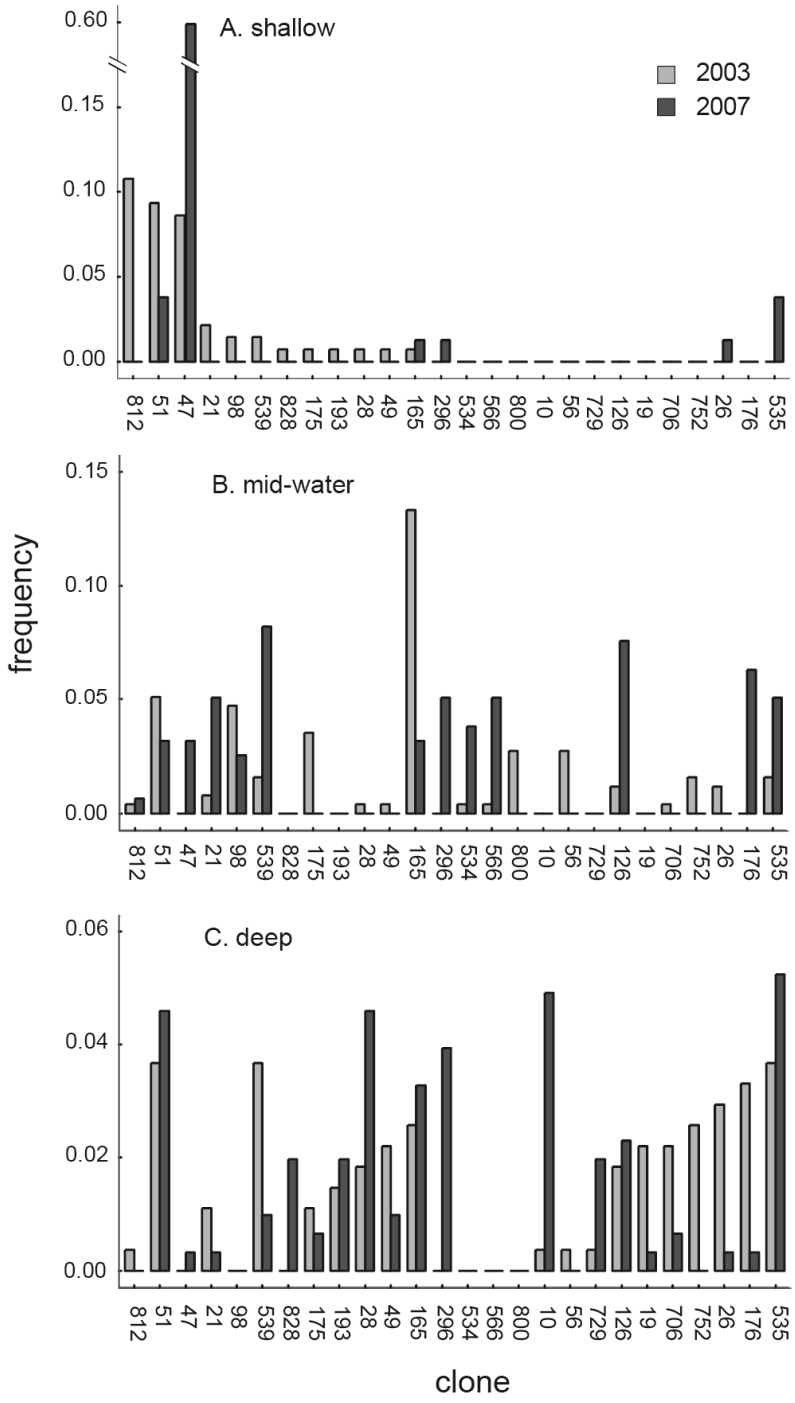


Figure 3. Frequencies of the most common clones among triploid individualsfrom the three habitats: A-shallow, B-mid-water, C-deep. Only clones that had a frequency >0.02 in any of the samples are shown. Frequencies in year 2003 are shown in light grey and 2007 in dark grey. Note the differences in y axis scale among habitats.

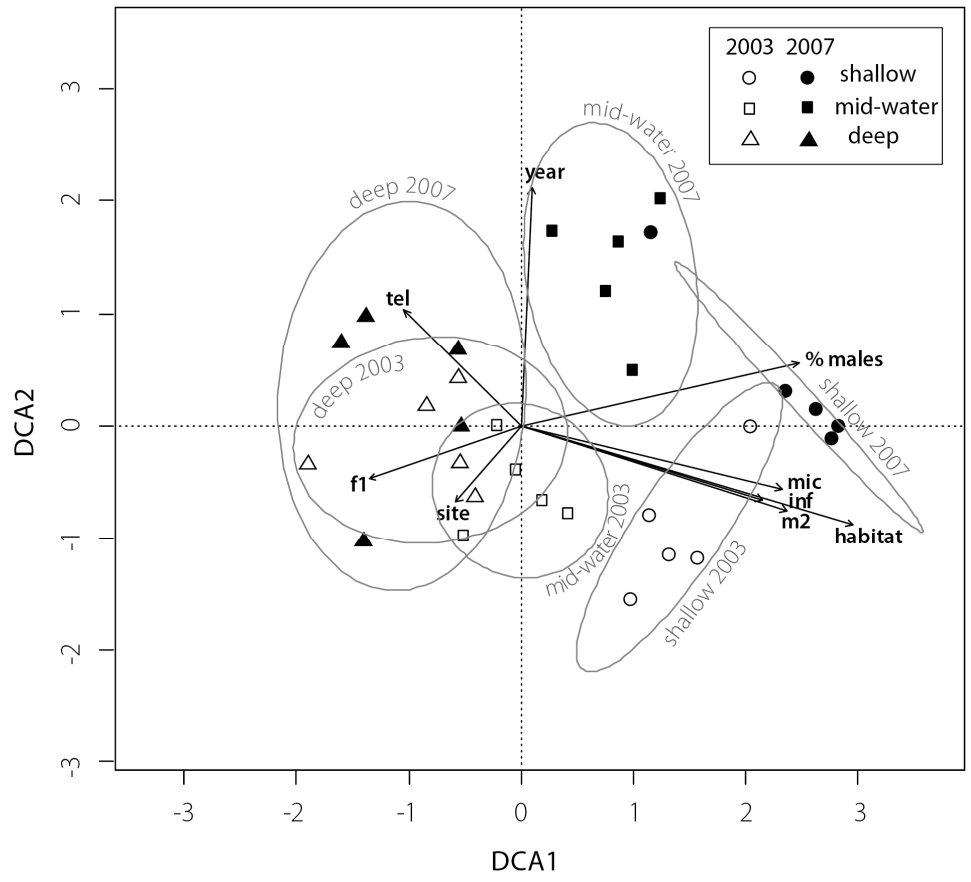


Figure 4. Scatterplot of first two axes of Detrended Correspondence Analysis (DCA) of clonal composition. Each point represents a sample from single habitat, site and year. Arrows represent vectors of factor averages of fitted structuring variables (year, habitat and site) and the variables obtained from samples screened for parasites: proportions of all trematode species (inf), *Microphallus livelyei* (mic), *Notocotylus gippyensis* (m2), *Telogaster opisthorchis* (tel) and *Apatemon* sp. (f1) and the percentage of males. Ellipses depict 90% confidence intervals calculated for samples grouped by habitat and year.

## Genetic Identity

The distribution of pairwise identity values for rare asexual clones matched the distribution of sexuals (Kernel probability density distribution, sexuals: median=0.681, 5 percentile=0.392, 95 percentile=0.969; rare clones: median=0.636, 5 percentile=0.285, 95 percentile=0.986). The distribution of pairwise identity values for the common clones was narrower and shifted

towards higher values (median=0.807, 5 percentile=0.591, 95 percentile=1.022), indicating that genetic similarity was on average higher among the common clones than the average similarity among sexual or rare asexual genotypes (Figure 5).

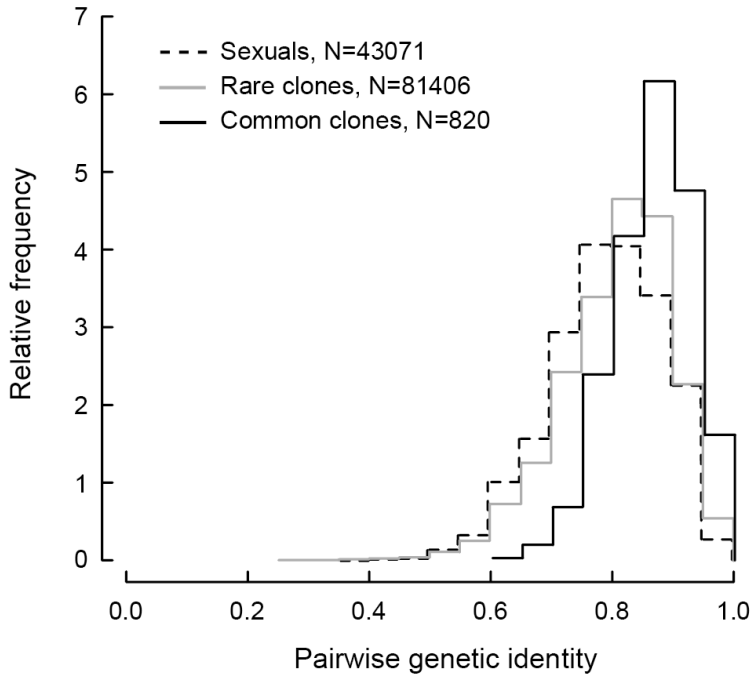


Figure 5. Relative frequency distributions of the pairwise genetic identity values within three groups of multilocus genotypes: sexuals (dashed), rare clones (grey) and common clones (black). *N* denotes the number of pairwise comparisons within each group.

We hypothesized that genotypes that are more dissimilar than resident snail genotypes (e.g. possible migrants) might have a selective advantage if parasites are locally adapted to resistance genotypes present in the local population. Under this "migrant-clone" scenario, we expected lower identity values between common clones and sexuals. In order to test this hypothesis, we compared calculated mean identity within and between groups of sexuals, common clones and rare clones to 1000 identity values drawn at random by permutating genotypes among these groups (Table 2). We found no support for this hypothesis in our data: the mean values of genetic identity between common clones and sexuals were either not significantly different or lower than the simulated mean in all comparisons (Table 2, C:S). The same was true for comparisons within rare clones (R:R), sexuals (S:S), and between rare clones and sexuals (R:S).

Some of the comparisons between common and rare clones had a significantly higher mean identity value than the simulated ones, and some yielded non-significant results (Table 2, C:R). The mean identity for within common clones comparisons (C:C) was significantly higher in all analyzed samples, meaning that common clones are more genetically similar to each other than to genotypes of the rare or sexual group.

Table 2. Mean and standard deviation of pairwise genetic-identity values (Band Sharing method) within and between groups of sexuals, common clones and rare clones. C:C- within common clones, R:R- within rare clones, S:S- within sexuals, C:R- between common and rare clones, C:S- between common clones and sexuals, R:S- between rare clones and sexuals. *N* denotes the number of common clones in a given habitat/year combination. For each of the samples within given habitat and year three replicates were calculated by randomly sampling *N* rare clone genotypes from the same habitat and year and *N* sexual genotypes from all sexual genotypes in the dataset. These observed mean identity values were compared to 1000 means obtained by permutating genotypes among groups. "NS" denotes *P* values that were not significant, *P*<0.05 denotes lower than simulated mean, *P*>0.95 (in bold) denotes higher than simulated mean.

| sample | mid-water 2003 | | | mid-water 2007 | | | deep 2003 | | | deep 2007 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 15 | | | 13 | | | 18 | | | 11 | | |
| | mean | SD | *P* | mean | SD | *P* | mean | SD | *P* | mean | SD | *P* |
| | 0.88 | 0.07 | **1** | 0.86 | 0.06 | **0.97** | 0.87 | 0.06 | **0.995** | 0.87 | 0.07 | **0.983** |
| C:C* | " | " | **0.998** | " | " | **1** | " | " | **1** | " | " | **0.993** |
| | " | " | **0.997** | " | " | **0.994** | " | " | **1** | " | " | **0.992** |
| | 0.84 | 0.06 | NS | 0.81 | 0.1 | NS | 0.85 | 0.06 | NS | 0.8 | 0.09 | NS |
| R:R | 0.84 | 0.06 | NS | 0.81 | 0.07 | NS | 0.8 | 0.08 | NS | 0.81 | 0.08 | NS |
| | 0.84 | 0.07 | NS | 0.81 | 0.1 | NS | 0.81 | 0.08 | NS | 0.85 | 0.07 | NS |
| | 0.82 | 0.08 | NS | 0.83 | 0.08 | NS | 0.81 | 0.09 | 0.036 | 0.8 | 0.08 | NS |
| S:S | 0.79 | 0.09 | 0.002 | 0.78 | 0.08 | NS | 0.8 | 0.09 | NS | 0.79 | 0.09 | NS |
| | 0.8 | 0.09 | 0.028 | 0.8 | 0.09 | NS | 0.78 | 0.09 | 0.009 | 0.82 | 0.09 | NS |
| | 0.86 | 0.06 | **0.998** | 0.82 | 0.08 | NS | 0.86 | 0.06 | **1** | 0.83 | 0.09 | NS |
| C:R | 0.87 | 0.07 | 1 | 0.82 | 0.08 | NS | 0.84 | 0.07 | **0.99** | 0.82 | 0.08 | **0.951** |
| | 0.86 | 0.07 | **0.999** | 0.84 | 0.08 | **0.985** | 0.84 | 0.07 | **0.998** | 0.86 | 0.06 | **0.991** |
| | 0.82 | 0.07 | 0.009 | 0.82 | 0.08 | NS | 0.81 | 0.08 | 0.006 | 0.82 | 0.09 | NS |
| C:S | 0.82 | 0.08 | NS | 0.8 | 0.08 | NS | 0.81 | 0.08 | NS | 0.78 | 0.09 | 0.009 |
| | 0.82 | 0.07 | 0.032 | 0.78 | 0.08 | 0.008 | 0.81 | 0.07 | NS | 0.81 | 0.08 | 0.008 |
| | 0.82 | 0.07 | 0.015 | 0.82 | 0.08 | NS | 0.81 | 0.08 | <0.001 | 0.8 | 0.09 | NS |
| R:S | 0.81 | 0.07 | 0.004 | 0.8 | 0.08 | NS | 0.79 | 0.09 | 0.002 | 0.79 | 0.09 | NS |
| | 0.81 | 0.07 | 0.026 | 0.79 | 0.1 | NS | 0.79 | 0.08 | <0.001 | 0.82 | 0.06 | NS |

*For C:C comparisons the replicates are not independent, as they contain the same common clones. Therefore, we report estimates for one draw only.

**Discussion**

The main aim of our study was to examine the temporal stability of genetic structure for a mixed population of sexual and asexual host snails. We predicted that the temporal dynamics should be less pronounced in deep-water habitat of the lake, where coevolving parasites are rare. Our results support this prediction: the clonal structure of the deep-water habitat did not change between the two time points over the 4-year interval analyzed in our study. For the shallow and mid-water habitats, where coevolving parasites are common, we found temporal fluctuation in the frequency of sexual individuals. Most importantly, we found significant changes in the structure of the asexual population over time in shallow and mid-water habitats. In addition, we found that clonal diversity and evenness changed in shallow habitat over time.

Our study system, *Potamopyrgus antipodarum* in Lake Alexandrina, is among the few natural systems where temporal dynamics and ecological drivers of clonal structure can be studied using reference data from earlier studies. In 1996, Fox and colleagues documented a strong clonal structure across a habitat specific depth-cline in Lake Alexandrina. The same study also showed that a clinal pattern was not detectable in the coexisting sexual population, from which the local clones are derived. At the same time, a series of studies by Jokela and colleagues (Jokela & Lively, 1995a; Jokela & Lively, 1995b) documented similarly strong habitat-specific clines in both frequency of sexual individuals and parasite prevalence, such that where parasites were common the sexual lineages dominated. Over a next decade, these studies were accompanied with a series of studies showing that the parasite populations show strong lake-specific local adaptation (reviewed by Lively *et al.*, 2004) and that common clones become highly susceptible to infection by parasites (Dybdahl & Lively, 1995a; Jokela *et al.*, 2009; Koskella & Lively, 2009). The first study to show how these processes come together in natural populations, and how they lead to rapid changes in the structure of clonal populations, was published by Jokela *et al. (*2009), where they documented changes in the clonal structure of shallow and mid-water habitats between 1994 and 6 (mid-water) or 9 (shallow) years after. This study also showed that common clones are resistant when they first become common, but they later become highly susceptible and decline in frequency after the parasites adapt to infect them.

Here we present an independent follow-up study to these studies. The novel approach we took in this study comes from analyzing the structure of the asexual population using multivariate analysis (the DCA ordination technique), which allows for analysis of the change at a level of the whole asexual population, not only at the level of individual clones. The second novel aspect of the study is that we analyzed the changes in the clonal structure also in the deep habitat, which has not been done before. The addition of this low-parasite habitat allowed us to evaluate the role of parasite-mediated selection in changing the clonal structure of populations by directly comparing the changes in clonal structure in habitats that differ in parasite pressure. Similar to earlier genetic studies, we found that clonal diversity was very high: roughly every third asexual individual was a new genotype, which is a close match to 0.27 new genotypes per genotyped asexual individuals reported in an earlier study by Fox *et al.* (1996). Comparing years 2003 and 2007, we observed a dramatic change in clonal diversity for the shallow habitat; the samples from 2007 showed a strong dominance of a single clonal genotype (Figure 2, Figure 3). In the other two habitats (mid-water and deep), the clonal richness, heterogeneity, and evenness indexes changed very little between the study years, and were very similar to those reported in Fox *et al.* (1996). The data reported in the Fox *et al.* (1996) was collected in 1994, which is nine and thirteen years before our surveys. This corresponds to at least an equal number of snail generations, based on estimates of reproductive rates of snails in field enclosures (D. Paczesniak, unpublished data). In other words, there should have been enough time for clonal diversity to change if the system were transitory.

One possible explanation for the high level of observed clonal diversity is a constant influx of new clonal genotypes from the coexisting sexual population. It is known that asexual *Potamopyrgus* clones are derived from their sexual counterparts (Dybdahl & Lively, 1995b); however the rate of emergence of new asexual lineages is presently not known (Neiman *et al.*, 2005). We found no evidence that high proportions of co-existing sexuals would have boosted clonal diversity, as clonal diversity was very high in the deep habitat, where sexuals are rare; and there was no consistent pattern in the relationship between the frequency of sexuals and clonal diversity in shallow and mid-water habitats. These findings are consistent with an earlier study by King *et al.* (2011a), which showed that genetic diversity of *P. antipodarum* clones in stream populations was not correlated with the frequency of co-existing sexuals.

This suggests that the establishment rate of new clones cannot be predicted from the frequency of sexual females in the population. Rather, in the study by King *et al.* (2011a), clonal diversity was higher in stream populations where the prevalence of virulent trematode parasites was high, suggesting that the local clonal diversity is maintained by parasite-mediated antagonistic coevolution.

The high diversity of the clonal population in the deep habitat of Lake Alexandrina may look as inconsistent with this pattern, and the maintenance of clonal diversity in the low parasite-risk environment calls for an explanation. The problem we have in evaluating clonal diversity among ecologically different lake habitats (not among stream populations as done in King *et al.* (2011a) is that the standing diversity is not affected only by the rate of emergence of clones, but also by the rate of extinction of clones. If the processes that promote extinction of clonal lineages are habitat specific (e.g. high parasite pressure), the diversity patterns among habitats may be difficult to interpret. For example, it is possible that the apparently high clonal diversity in the deep habitat is a result of weak temporal dynamics that reduce the extinction rates of clones to much lower than in the shallower habitats, which allows accumulation of clonal diversity to high levels. Nevertheless, the apparently stable maintenance of high clonal diversity in the deep habitat requires attention, as it seems to stand against ecological and evolutionary theories of coexistence. One possibility is that the clonal diversity in the deep is maintained by parasite species that cycle through eels (e.g. *Telogaster opisthorchis* and *Stegodexamene anguillae*), which are associated with deep habitats. The selection for the maintenance of clonal diversity need not be as strong as selection for maintenance of sexual reproduction (Lively & Howard, 1994) , therefore, lower infection frequencies might suffice. For Lake Alexandrina the hypothesis remains untested, because since the Tekapo Dam was built in 1963, the eel population has been declining, making it difficult to evaluate the present selection pressure by eel parasites in the deep.. Testing this hypothesis would require a comparative test of clonal diversity in deep habitats among lakes that differ by frequency of eel parasites.

Shifts in the genetic composition of a population can be caused by selection or drift. In this case, several lines of evidence argue against drift as an explanation for the observed changes. First, the temporal stability of allele frequencies in the co-existing sexual population suggests that the effects of drift are negligible (see Supporting Information, Table S3). This is not

surprising considering that the estimate of mean population density of *P. antipodarum* in Lake Alexandrina is over 5600 ind/$m^2$ (K. Kopp, unpublished data), which would bring the census population size to $10^9$ individuals in the mid-water habitat alone (given that average width of mid-water habitat is 11m, and the length of the shoreline of Lake Alexandrina is 17 km). Second, the fact that rapid dynamics in asexuals were not observed in the deep habitat speaks against drift as an explanation. Population density is generally very high in all habitats, especially in the mid-water, where changes in clonal structure were observed. Third, if drift were important, we would expect more spatial structure in the clonal population as the snails are unlikely to mix across long distances during their life-span.

Rapid changes in the composition of common clones in the shallow and mid-water habitats are consistent with patterns expected under strong frequency-dependent selection by parasites (Jokela *et al.*, 2009). Parasite pressure in Lake Alexandrina is the highest in the shallow habitat and decreases with depth (Jokela & Lively, 1995b), this is also what we found in this study (Figure 4, Supporting Information Table S6), and that this pattern has been shown to be stable over time (Jokela *et al.*, 2009). In addition, the shallow habitat has been shown to be a coevolutionary hotspot, and the deep habitat a coevolutionary coldspot (King *et al.*, 2009; 2011b). The possibility for the snail-trematode coevolution to operate in this system even without direct transmission of the parasite may be due to fact that these short-lived (weeks, not months) adult parasites most likely cycle through young birds, in which the humoral immune response is weaker than in adults (Butler & McGraw, 2012). During the first weeks after hatching the ducklings are less likely to move for long distances, but rather staying at the same area of the lake, and this might be sufficient to cycle these parasites locally.

One of the more interesting results from this study is that the sexual population in the deep habitat, which was estimated as 10% in 1994 by Fox *et al.* (1996) had declined to near zero per cent by 2003. This result shows that clones can outcompete sexuals in the absence of parasites, and that the long-term persistence of coexisting sexual and asexual populations can change. Our results also indicate that the clonal dynamics in the deep between 2003 and 2007 are not as rapid as in the shallow, suggesting that selective factors are different. We also found that 18 out of 23 common clones (frequency above 1.5%) of the deep samples in 2003 or 2007 were the same as those found in 1994 (J. Jokela, unpublished).

Clonal dynamics of asexual populations in response to environmental factors have been previously reported. For example Carvalho & Crisp (1987) found seasonal changes in the frequencies of clones of the freshwater crustacean *Daphnia magna* to be related to changing environmental conditions: temperature and population density. In addition, Vorburger (2006) found rapid temporal dynamics (within one year) in frequencies of clones in a population of aphid *Myzus persicae*, which can be related to seasonal availability of host plants. Little & Ebert (1999) observed temporal changes in clonal composition of *Daphnia* populations, which in some cases were consistent with parasite-mediated selection. While in our case it is difficult to explain the temporal change in the common clones of shallow and mid-water habitats without appealing to fluctuating selection, several related questions remain unanswered. The parasite theory alone does not provide a mechanism for extinction of clones, which is needed to prevent accumulation of high diversity of rare clones. Highly diverse clonal population is predicted to capitalize on the cost of males and replace the sexual population with a diverse clonal population (Lively & Howard, 1994). Parasites are inferred to explain the decline of the common clones through erosion of their fitness advantage by parasite adaptation (Jokela *et al.*, 2009), but for a large population of rare clones the argument focusing on common clones may not suffice to counter the cost of males. A possible explanation may lie in differences in fitness among clones, which are initially derived from their sexual ancestors. Not all sexual genotypes have equal fitness, and it is known that clones differ, for example, in their reproductive output (Jokela *et al.*, 2003, Paczesniak et al. unpublished data ). It is likely that a randomly derived triploid genotype has a lower average fitness than the best sexual lineages. In a threshold zone where an average clone just matches the fitness of best sexual lineages, the clones would not rapidly increase in frequency and attract parasite coevolution, but might persist in head-to-head competition with the sexuals. On the other hand, those clones that are derived from the very best sexual lineages would increase rapidly and may become disproportionately infected after becoming common, and driven down in frequency as depicted in Jokela *et al.* (2009). As these clones age, the mutational processes (Mullers ratchet, mutation accumulation) would inevitably erode their fitness, although it is difficult to estimate how long this takes. These, unfortunately rather speculative, arguments call for empirical work aimed at measuring the fitness distribution of asexual genotypes in ecologically relevant conditions and comparing that to the coexisting

sexuals. Stable coexistence of rare clones and sexuals would require that the best sexual lineages can successfully compete against the average clonal lineages.

Under the parasite hypothesis for maintenance of sex, parasites adapt to common genotypes through Red Queen type coevolution. In parasite-ridden world, it is advantageous to carry resistance alleles and it has been demonstrated that indeed the recently emerged common clones are very resistant to parasites (Jokela *et al.*, 2009). In this system, parasites are generally locally adapted to the host population of the lake, therefore migrant snails would be expected to carry resistance alleles that are novel to the population. To test the idea that emerging common clones would in fact be migrants, we calculated the genetic similarities between different groups of genotypes. Common clones should have been genetically more dissimilar to an average genotype found in the lake if they were migrants and became common because they enjoyed the advantage of rare resistance alleles. We found no support for this hypothesis. An earlier population genetic study between the lake populations showing high genetic structure and low migration rates supports this conclusion (Dybdahl & Lively, 1995b). Interestingly, we observed the opposite pattern: common clones were on average more similar to each other than to sexuals or rare clones. This reduction in diversity may be due to either a genetic bottleneck (if common clones are derived through clonal divergence and share recent common asexual ancestor), or due to selection (if a selected locus is linked to any of the allozyme loci we used as presumably neutral markers).

In conclusion, our study reveals very pronounced temporal dynamics in clonal structure in habitats where the snail population is most likely to be target for genotype-specific frequency-dependent selection by parasites. We do not see such dynamics in the habitat where such selection is unlikely. Our results thus support the hypothesis that adaptation by parasites is a key factor preventing the fixation of high fitness asexual genotypes and consequent extinction of the sexual population. In fact, our survey data reveals that in the deep habitat, where parasite pressure is weak, the sexual population has gone virtually extinct during the last two decades.

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**Literature cited**

Altermatt, F., Hottinger, J. & Ebert, D. 2007. Parasites promote host gene flow in a metapopulation. *Evol. Ecol.* **21:** 561-575.

Arnaud-Haond, S. & Belkhir, K. 2007. GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Mol. Ecol. Notes* **7:** 15-17.

Arnaud-Haond, S., Duarte, C.M., Alberto, F. & Serrao, E.A. 2007. Standardizing methods to address clonality in population studies. *Mol. Ecol.* **16:** 5115-5139.

Butler, M.W. & McGraw, K.J. 2012. Developmental immune history affects adult immune function but not carotenoid-based ornamentation in mallard ducks. *Funct. Ecol.* **26:** 406-415.

Carvalho, G.R. & Crisp, D.J. 1987. The clonal ecology of *Daphnia magna* (Crustacea: Cladocera): I. Temporal changes in the clonal structure of a natural population. *J. Anim. Ecol.* **56:** 453-468.

Dybdahl, M.F. & Lively, C.M. 1995a. Host-parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). *Proc. R. Soc. B* **260:** 99-103.

Dybdahl, M.F. & Lively, C.M. 1995b. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). *J. Evol. Biol.* **8:** 385-398.

Dybdahl, M.F. & Lively, C.M. 1996. The geography of coevolution: comparative population structures for a snail and its trematode parasite. *Evolution* **50:** 2264-2275.

Fisher, R.A. 1930. Sexual reproduction and sexual selection. In: *The genetical theory of natural selection*, pp. 121-145. Clarendon Press, Oxford.

Fox, J.A., Dybdahl, M.F., Jokela, J. & Lively, C.M. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). *Evolution* **50:** 1541-1548.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *J. Hered.* **86:** 485-486.

Haldane, J.B.S. 1924. A mathematical theory of natural and artificial selection. Part I. *Transactions of the Cambridge Philosophical Society* **23:** 19-41.

Haldane, J.B.S. 1949. Disease and evolution. *La Ricerca Scientifica (Supplement)* **19:** 68-76.

Hamilton, W.D. 1980. Sex versus non-sex versus parasite. *Oikos* **35:** 282-290.
Hill, M.O. & Gauch, H.G. 1980. Detrended correspondence analysis: an improved ordination technique. *Vegetatio* **42:** 47-58.
Jaenike, J. 1978. An hypothesis to account for the maintenance of sex within populations. *Evol. Theor.* **3:** 191-194.
Jokela, J. & Lively, C.M. 1995a. Parasites, sex, and early reproduction in a mixed population of freshwater snails. *Evolution* **49:** 1268-1271.
Jokela, J. & Lively, C.M. 1995b. Spatial variation in infection by digenetic trematodes in a population of freshwater snails (*Potamopyrgus antipodarum*). *Oecologia* **103:** 509-517.
Jokela, J., Lively, C.M., Fox, J.A. & Dybdahl, M.F. 1997a. Flat reaction norms and "frozen" phenotypic variation in clonal snails (*Potamopyrgus antipodarum*). *Evolution* **51:** 1120-1129.
Jokela, J., Lively, C.M., Dybdahl, M.F. & Fox, J.A. 1997b. Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. *Ecology* **78:** 452-460.
Jokela, J., C. M. Lively, M. F. Dybdahl & Fox, J.A. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. *Biol. J. Linn. Soc.* **79:** 165-181.
Jokela, J., Dybdahl, M.F. & Lively, C.M. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *Am. Nat.* **174:** S43-S53.
King, K.C., Delph, L.F., Jokela, J. & Lively, C.M. 2009. The geographic mosaic of sex and the Red Queen. *Curr. Biol.* **19:** 1438-1441.
King, K.C., Jokela, J. & Lively, C.M. 2011a. Parasites, sex and clonal diversity in natural snail populations. *Evolution* **65:** 1474-1481.
King, K.C., Delph, L.F., Jokela, J. & Lively, C.M. 2011b. Coevolutionary hotspots and coldspots for host sex and parasite local adaptation in a snail-trematode interaction. *Oikos* **120:** 1335-1340.
Koskella, B. & Lively, C.M. 2009. Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution* **63:** 2213-2221.
Little, T.J. & Ebert, D. 1999. Associations between parasitism and host genotype in natural populations of *Daphnia* (Crustacea: Cladocera). *J. Anim. Ecol.* **68:** 134-149.
Lively, C.M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature* **328:** 519-521.
Lively, C.M. & Howard, R.S. 1994. Selection by parasites for clonal diversity and mixed mating. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **346:** 271-281.
Lively, C.M., Dybdahl, M.F., Jokela, J., Osnas, E.E. & Delph, L.F. 2004. Host sex and local adaptation by parasites in a snail-trematode interaction. *Am. Nat.* **164:** S6-S18.
Ludwig, J.A. & Reynolds, J.F. 1988. *Statistical ecology: a primer on methods and computing*. John Wiley & Sons, New York.
Miller, M.P. 1997. Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.
Neiman, M., Jokela, J. & Lively, C.M. 2005. Variation in asexual lineage age in *Potamopyrgus antipodarum*, a New Zealand snail. *Evolution* **59:** 1945-1952.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. 2010. Vegan: Community Ecology Package. R package version 1.17-4. http://CRAN.R-project.org/package=vegan.

Pongratz, N., Sharbel, T.F., Beukeboom, L.W. & Michiels, N.K. 1998. Allozyme variability in sexual and parthenogenetic freshwater planarians: evidence for polyphyletic origin of parthenogenetic lineages through hybridization with coexisting sexuals. *Heredity* **81:** 38-47.

R Development Core Team 2009. R: A language and environment for statistical computing R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Scheiner, S.M. & Yampolsky, L.Y. 1998. The evolution of *Daphnia pulex* in a temporally varying environment. *Genet. Res.* **72:** 25-37.

Simon, J.C., Delmotte, F., Rispe, C. & Crease, T. 2003. Phylogenetic relationships between parthenogens and their sexual relatives: the possible routes to parthenogenesis in animals. *Biol. J. Linn. Soc.* **79:** 151-163.

Simpson, E.H. 1949. Measurement of diversity. *Nature* **163:** 688-688.

Spitze, K. 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex*: temporal pattern of population diversity, fitness, and mean life history. *Evolution* **45:** 82-92.

Stoddart, J.A. & Taylor, J.F. 1988. Genotypic diversity - estimation and prediction in samples. *Genetics* **118:** 705-711.

Tomiuk, J., Guldbrandtsen, B. & Loeschcke, V. 2009. Genetic similarity of polyploids: a new version of the computer program POPDIST (version 1.2.0) considers intraspecific genetic differentiation. *Mol. Ecol. Resour.* **9:** 1364-1368.

Vanoverbeke, J. & De Meester, L. 2010. Clonal erosion and genetic drift in cyclical parthenogens - the interplay between neutral and selective processes. *J. Evol. Biol.* **23:** 997-1012.

Vorburger, C. 2006. Temporal dynamics of genotypic diversity reveal strong clonal selection in the aphid *Myzus persicae*. *J. Evol. Biol.* **19:** 97-107.

Wallace, C. 1992. Parthenogenesis, sex and chromosomes in *Potamopyrgus*. *J. Molluscan Stud.* **58:** 93-107.

Ward, J. & Talbot, J. 1984. Distribution of aquatic macrophytes in Lake Alexandrina, New Zealand. *N. Z. J. Mar. Freshw. Res.* **18:** 211-220.

Winterbourn, M.J. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). *Malacologia* **10:** 283-321.

Wolinska, J. & Spaak, P. 2009. The cost of being common: Evidence from natural *Daphnia* populations. *Evolution* **63:** 1893-1901.

## Supporting Information

Table S1. GPS coordinates of the sampling sites.

| site | latitude | longtitude |
|---|---|---|
| West Bay | -43.949192° | 170.440386° |
| 2nd fence | -43.959306° | 170.442194° |
| 1st fence | -43.963611° | 170.442331° |
| Camp | -43.965611° | 170.442426° |
| East Side | -43.961786° | 170.455203° |

Table S2. Numbers and percentages of sexual, asexual and unassigned individuals within samples. "Unassigned ploidy" denotes the individuals for which ploidy level could not be assigned. The column "triploid, unkn. clonal identity" lists individuals for which we obtained incomplete genotype and could not unambiguously assign as members of a specific clone.

| | | | sexual | | asexual | | unassigned ploidy | | triploid, unkn. clonal identity | | number of clones |
|---|---|---|---|---|---|---|---|---|---|---|---|
| year | habitat | site | N | % | N | % | N | % | N | % | |
| 2003 | shallow | 1st fence | 30 | 45.5 | 36 | 54.5 | 0 | 0.0 | 2 | 3.0 | 29 |
| 2003 | shallow | 2nd fence | 25 | 37.9 | 41 | 62.1 | 0 | 0.0 | 2 | 3.0 | 23 |
| 2003 | shallow | Camp | 36 | 54.5 | 30 | 45.5 | 0 | 0.0 | 5 | 7.6 | 20 |
| 2003 | shallow | East side | 41 | 62.1 | 25 | 37.9 | 0 | 0.0 | 0 | 0.0 | 21 |
| 2003 | shallow | West bay | 42 | 63.6 | 18 | 27.3 | 6 | 9.1 | 2 | 3.0 | 14 |
| 2003 | mid-water | 1st fence | 6 | 9.1 | 59 | 89.4 | 1 | 1.5 | 3 | 4.5 | 28 |
| 2003 | mid-water | 2nd fence | 8 | 12.1 | 58 | 87.9 | 0 | 0.0 | 11 | 16.7 | 32 |
| 2003 | mid-water | Camp | 3 | 4.5 | 63 | 95.5 | 0 | 0.0 | 8 | 12.1 | 38 |
| 2003 | mid-water | East side | 6 | 9.1 | 60 | 90.9 | 0 | 0.0 | 10 | 15.2 | 36 |
| 2003 | mid-water | West bay | 15 | 22.7 | 51 | 77.3 | 0 | 0.0 | 4 | 6.1 | 38 |
| 2003 | deep | 1st fence | 1 | 1.5 | 65 | 98.5 | 0 | 0.0 | 14 | 21.2 | 28 |
| 2003 | deep | 2nd fence | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 9 | 13.6 | 42 |
| 2003 | deep | Camp | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 12 | 18.2 | 38 |
| 2003 | deep | East side | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 18 | 27.3 | 41 |
| 2003 | deep | West bay | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 4 | 6.1 | 47 |
| 2007 | shallow | 1st fence | 42 | 63.6 | 24 | 36.4 | 0 | 0.0 | 2 | 3.0 | 9 |
| 2007 | shallow | 2nd fence | 42 | 63.6 | 22 | 33.3 | 2 | 3.0 | 3 | 4.5 | 6 |
| 2007 | shallow | Camp | 46 | 69.7 | 20 | 30.3 | 0 | 0.0 | 1 | 1.5 | 6 |
| 2007 | shallow | East side | 56 | 84.8 | 10 | 15.2 | 0 | 0.0 | 0 | 0.0 | 4 |
| 2007 | shallow | West bay | 54 | 81.8 | 9 | 13.6 | 3 | 4.5 | 0 | 0.0 | 8 |
| 2007 | mid-water | 1st fence | 32 | 48.5 | 34 | 51.5 | 0 | 0.0 | 7 | 10.6 | 21 |
| 2007 | mid-water | 2nd fence | 26 | 39.4 | 39 | 59.1 | 1 | 1.5 | 3 | 4.5 | 25 |
| 2007 | mid-water | Camp | 31 | 47.0 | 34 | 51.5 | 1 | 1.5 | 4 | 6.1 | 27 |
| 2007 | mid-water | East side | 24 | 36.4 | 41 | 62.1 | 1 | 1.5 | 2 | 3.0 | 19 |
| 2007 | mid-water | West bay | 36 | 54.5 | 30 | 45.5 | 0 | 0.0 | 4 | 6.1 | 15 |
| 2007 | deep | 1st fence | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 9 | 13.6 | 41 |
| 2007 | deep | 2nd fence | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 5 | 7.6 | 37 |
| 2007 | deep | Camp | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 2 | 3.0 | 42 |
| 2007 | deep | East side | 0 | 0.0 | 66 | 100.0 | 0 | 0.0 | 6 | 9.1 | 46 |
| 2007 | deep | West bay | 0 | 0.0 | 65 | 98.5 | 1 | 1.5 | 2 | 3.0 | 40 |

Table S3. Sexual population allele frequencies and numbers of individuals (N) within samples for each locus. Weighted and unweighted averages across all samples are shown below.

| | | locus | AAT2 | | | | AAT1 | | | MPI | | | | 6PGD | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | allele | | 1 | 2 | 3 | | 1 | 2 | | 3 | 5 | 6 | | 3 | 4 | 5 |
| year | site | habitat | N | | | | N | | | N | | | | N | | | |
| 2003 | 1st fence | shallow | 29 | 0.379 | 0.621 | 0.000 | 30 | 0.933 | 0.067 | 30 | 0.883 | 0.117 | 0.000 | 30 | 0.650 | 0.283 | 0.067 |
| 2003 | 1st fence | mid-water | 6 | 0.500 | 0.500 | 0.000 | 6 | 0.833 | 0.167 | 4 | 1.000 | 0.000 | 0.000 | 6 | 0.583 | 0.417 | 0.000 |
| 2003 | 2nd fence | shallow | 25 | 0.520 | 0.480 | 0.000 | 25 | 0.980 | 0.020 | 25 | 0.860 | 0.140 | 0.000 | 25 | 0.440 | 0.540 | 0.020 |
| 2003 | 2nd fence | mid-water | 8 | 0.438 | 0.563 | 0.000 | 8 | 1.000 | 0.000 | 7 | 0.857 | 0.143 | 0.000 | 8 | 0.438 | 0.438 | 0.125 |
| 2003 | Camp | shallow | 35 | 0.529 | 0.471 | 0.000 | 36 | 0.931 | 0.069 | 35 | 0.914 | 0.086 | 0.000 | 34 | 0.574 | 0.368 | 0.059 |
| 2003 | Camp | mid-water | 3 | 0.500 | 0.500 | 0.000 | 3 | 1.000 | 0.000 | 3 | 1.000 | 0.000 | 0.000 | 3 | 0.500 | 0.500 | 0.000 |
| 2003 | East side | shallow | 40 | 0.388 | 0.613 | 0.000 | 41 | 0.927 | 0.073 | 41 | 0.915 | 0.085 | 0.000 | 41 | 0.585 | 0.317 | 0.098 |
| 2003 | East side | mid-water | 5 | 0.300 | 0.700 | 0.000 | 6 | 1.000 | 0.000 | 6 | 1.000 | 0.000 | 0.000 | 6 | 0.583 | 0.417 | 0.000 |
| 2003 | West bay | shallow | 39 | 0.359 | 0.641 | 0.000 | 42 | 0.976 | 0.024 | 42 | 0.881 | 0.119 | 0.000 | 40 | 0.550 | 0.413 | 0.038 |
| 2003 | West bay | mid-water | 13 | 0.423 | 0.577 | 0.000 | 15 | 0.900 | 0.100 | 14 | 0.893 | 0.107 | 0.000 | 15 | 0.600 | 0.367 | 0.033 |
| 2007 | 1st fence | shallow | 40 | 0.275 | 0.725 | 0.000 | 41 | 0.976 | 0.024 | 42 | 0.869 | 0.119 | 0.012 | 41 | 0.451 | 0.512 | 0.037 |
| 2007 | 1st fence | mid-water | 32 | 0.344 | 0.656 | 0.000 | 32 | 0.953 | 0.047 | 32 | 0.906 | 0.094 | 0.000 | 31 | 0.548 | 0.435 | 0.016 |
| 2007 | 2nd fence | shallow | 40 | 0.338 | 0.663 | 0.000 | 40 | 0.988 | 0.013 | 42 | 0.976 | 0.024 | 0.000 | 42 | 0.607 | 0.357 | 0.036 |
| 2007 | 2nd fence | mid-water | 26 | 0.327 | 0.673 | 0.000 | 25 | 0.960 | 0.040 | 26 | 0.981 | 0.000 | 0.019 | 26 | 0.596 | 0.365 | 0.038 |
| 2007 | Camp | shallow | 45 | 0.322 | 0.678 | 0.000 | 46 | 0.978 | 0.022 | 46 | 0.913 | 0.087 | 0.000 | 46 | 0.543 | 0.337 | 0.120 |
| 2007 | Camp | mid-water | 31 | 0.371 | 0.629 | 0.000 | 31 | 0.887 | 0.113 | 31 | 0.903 | 0.097 | 0.000 | 31 | 0.613 | 0.339 | 0.048 |
| 2007 | East side | shallow | 56 | 0.313 | 0.688 | 0.000 | 56 | 0.973 | 0.027 | 56 | 0.955 | 0.045 | 0.000 | 56 | 0.598 | 0.330 | 0.071 |
| 2007 | East side | mid-water | 23 | 0.348 | 0.652 | 0.000 | 23 | 0.935 | 0.065 | 24 | 0.854 | 0.146 | 0.000 | 24 | 0.521 | 0.354 | 0.125 |
| 2007 | West bay | shallow | 53 | 0.264 | 0.736 | 0.000 | 54 | 0.954 | 0.046 | 54 | 0.833 | 0.167 | 0.000 | 53 | 0.528 | 0.425 | 0.047 |
| 2007 | West bay | mid-water | 36 | 0.389 | 0.597 | 0.014 | 36 | 0.931 | 0.069 | 33 | 0.924 | 0.076 | 0.000 | 33 | 0.576 | 0.394 | 0.030 |
| | All weighted | | | 0.360 | 0.639 | 0.001 | | 0.953 | 0.047 | | 0.906 | 0.092 | 0.002 | | 0.559 | 0.384 | 0.057 |
| | All unweighted | | | 0.381 | 0.618 | 0.001 | | 0.951 | 0.049 | | 0.916 | 0.083 | 0.002 | | 0.554 | 0.395 | 0.050 |

| | | locus | IDH2 | | | IDH1 | | | | PEP-D | | | | PGM2 | | PGM1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | allele | | 2 | 3 | | 2 | 3 | 4 | | 2 | 3 | 4 | | 1 | 2 | | 1 | 2 |
| year | site | habitat | N | | | N | | | | N | | | | N | | | N | | |
| 2003 | 1st fence | shallow | 30 | 1.000 | 0.000 | 30 | 0.417 | 0.583 | 0.000 | 29 | 0.603 | 0.379 | 0.017 | 30 | 0.850 | 0.150 | 30 | 0.000 | 1.000 |
| 2003 | 1st fence | mid-water | 6 | 1.000 | 0.000 | 6 | 0.167 | 0.833 | 0.000 | 3 | 0.667 | 0.333 | 0.000 | 6 | 0.833 | 0.167 | 6 | 0.000 | 1.000 |
| 2003 | 2nd fence | shallow | 25 | 1.000 | 0.000 | 25 | 0.160 | 0.840 | 0.000 | 25 | 0.520 | 0.460 | 0.020 | 25 | 0.840 | 0.160 | 24 | 0.042 | 0.958 |
| 2003 | 2nd fence | mid-water | 8 | 1.000 | 0.000 | 5 | 0.200 | 0.800 | 0.000 | 8 | 0.750 | 0.250 | 0.000 | 8 | 0.875 | 0.125 | 8 | 0.063 | 0.938 |
| 2003 | Camp | shallow | 36 | 0.986 | 0.014 | 32 | 0.359 | 0.625 | 0.016 | 35 | 0.600 | 0.386 | 0.014 | 35 | 0.814 | 0.186 | 35 | 0.014 | 0.986 |
| 2003 | Camp | mid-water | 3 | 1.000 | 0.000 | 3 | 0.167 | 0.833 | 0.000 | 2 | 0.250 | 0.500 | 0.250 | 3 | 0.833 | 0.167 | 3 | 0.000 | 1.000 |
| 2003 | East side | shallow | 40 | 0.988 | 0.013 | 40 | 0.350 | 0.650 | 0.000 | 39 | 0.628 | 0.359 | 0.013 | 41 | 0.841 | 0.159 | 41 | 0.000 | 1.000 |
| 2003 | East side | mid-water | 6 | 1.000 | 0.000 | 6 | 0.417 | 0.583 | 0.000 | 6 | 0.750 | 0.250 | 0.000 | 6 | 0.667 | 0.333 | 6 | 0.167 | 0.833 |
| 2003 | West bay | shallow | 42 | 1.000 | 0.000 | 39 | 0.308 | 0.692 | 0.000 | 41 | 0.634 | 0.341 | 0.024 | 40 | 0.675 | 0.325 | 42 | 0.012 | 0.988 |
| 2003 | West bay | mid-water | 15 | 1.000 | 0.000 | 14 | 0.500 | 0.464 | 0.036 | 11 | 0.727 | 0.273 | 0.000 | 15 | 0.833 | 0.167 | 14 | 0.000 | 1.000 |
| 2007 | 1st fence | shallow | 41 | 0.976 | 0.024 | 41 | 0.256 | 0.744 | 0.000 | 41 | 0.537 | 0.463 | 0.000 | 41 | 0.817 | 0.183 | 41 | 0.012 | 0.988 |
| 2007 | 1st fence | mid-water | 32 | 0.953 | 0.047 | 31 | 0.323 | 0.677 | 0.000 | 31 | 0.468 | 0.532 | 0.000 | 32 | 0.750 | 0.250 | 32 | 0.047 | 0.953 |
| 2007 | 2nd fence | shallow | 42 | 0.988 | 0.012 | 40 | 0.338 | 0.663 | 0.000 | 42 | 0.548 | 0.440 | 0.012 | 42 | 0.845 | 0.155 | 42 | 0.012 | 0.988 |
| 2007 | 2nd fence | mid-water | 26 | 0.981 | 0.019 | 25 | 0.300 | 0.700 | 0.000 | 26 | 0.423 | 0.577 | 0.000 | 26 | 0.923 | 0.077 | 26 | 0.000 | 1.000 |
| 2007 | Camp | shallow | 46 | 0.978 | 0.022 | 45 | 0.356 | 0.644 | 0.000 | 46 | 0.500 | 0.500 | 0.000 | 46 | 0.793 | 0.207 | 46 | 0.022 | 0.978 |
| 2007 | Camp | mid-water | 30 | 0.983 | 0.017 | 27 | 0.315 | 0.685 | 0.000 | 29 | 0.500 | 0.500 | 0.000 | 31 | 0.823 | 0.177 | 31 | 0.016 | 0.984 |
| 2007 | East side | shallow | 56 | 0.982 | 0.018 | 56 | 0.482 | 0.518 | 0.000 | 56 | 0.500 | 0.500 | 0.000 | 56 | 0.652 | 0.348 | 56 | 0.009 | 0.991 |
| 2007 | East side | mid-water | 24 | 0.958 | 0.042 | 23 | 0.304 | 0.696 | 0.000 | 24 | 0.479 | 0.521 | 0.000 | 24 | 0.833 | 0.167 | 24 | 0.063 | 0.938 |
| 2007 | West bay | shallow | 54 | 0.972 | 0.028 | 53 | 0.264 | 0.736 | 0.000 | 54 | 0.537 | 0.463 | 0.000 | 54 | 0.694 | 0.306 | 54 | 0.000 | 1.000 |
| 2007 | West bay | mid-water | 36 | 0.944 | 0.056 | 36 | 0.222 | 0.778 | 0.000 | 36 | 0.556 | 0.444 | 0.000 | 36 | 0.764 | 0.236 | 36 | 0.028 | 0.972 |
| | All weighted | | | 0.981 | 0.019 | | 0.326 | 0.672 | 0.002 | | 0.547 | 0.446 | 0.007 | | 0.784 | 0.216 | | 0.018 | 0.982 |
| | All unweighted | | | 0.984 | 0.016 | | 0.310 | 0.687 | 0.003 | | 0.559 | 0.424 | 0.018 | | 0.798 | 0.202 | | 0.025 | 0.975 |

Table S4. *P* values of exact tests for agreement with Hardy-Weinberg equilibrium. Only values of $P < 0.05$ are shown (180 tests performed).

| year | site | habitat | locus | *P* |
|---|---|---|---|---|
| 2003 | East side | shallow | PEP-D | 0.0002 * |
| 2003 | 1st fence | shallow | PEP-D | 0.0010 |
| 2003 | Camp | shallow | PEP-D | 0.0013 |
| 2003 | 2nd fence | shallow | PEP-D | 0.0021 |
| 2003 | West bay | shallow | PEP-D | 0.0090 |
| 2003 | West bay | mid-water | IDH1 | 0.0014 |
| 2007 | 2nd fence | shallow | IDH1 | 0.0277 |
| 2003 | West bay | mid-water | AAT2 | 0.0236 |
| 2007 | 2nd fence | mid-water | AAT1 | 0.0246 |
| 2007 | East side | shallow | AAT1 | 0.0262 |
| 2007 | Camp | mid-water | AAT1 | 0.0278 |
| 2007 | 2nd fence | shallow | 6PGD | 0.0431 |

* $P < 0.05$ after sequential Bonferroni correction

Table S5. Matrix of pairwise Fst values between sexual samples. In bold comparisons for which P-values > 0.05. P-values were obtained after 3800 permutations.

| | 2003 1st fence mid-wate | 2003 2nd fence shallow | 2003 2nd fence mid-wate | 2003 Camp shallow | 2003 Camp mid-water | 2003 East side shallow | 2003 East side mid-wate | 2003 West Bay shallow | 2003 West Bay mid-wate | 2007 1st fence shallow | 2007 1st fence mid-wate | 2007 2nd fence shallow | 2007 2nd fence mid-wate | 2007 Camp shallow | 2007 Camp mid-water | 2007 East side shallow | 2007 East side mid-wate | 2007 West Bay shallow | 2007 West Bay mid-wate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2003 1st fence shallow | 0.01 | **0.05** | 0.00 | 0.00 | -0.02 | -0.01 | -0.01 | 0.01 | -0.02 | **0.03** | 0.00 | 0.00 | 0.00 | 0.00 | -0.01 | 0.01 | 0.00 | **0.02** | 0.01 |
| 2003 1st fence mid-water | | -0.02 | -0.03 | -0.02 | -0.06 | -0.02 | 0.00 | 0.00 | 0.01 | 0.01 | -0.01 | -0.01 | -0.01 | 0.00 | -0.03 | 0.04 | -0.02 | 0.01 | -0.03 |
| 2003 2nd fence shallow | | | -0.03 | 0.02 | -0.08 | **0.03** | 0.04 | 0.03 | 0.05 | 0.02 | 0.02 | **0.04** | **0.04** | **0.04** | 0.03 | **0.09** * | 0.02 | **0.04** | 0.01 |
| 2003 2nd fence mid-water | | | | -0.01 | -0.09 | -0.02 | -0.02 | -0.01 | 0.00 | -0.02 | -0.02 | -0.01 | -0.01 | -0.02 | -0.02 | 0.04 | -0.04 | -0.01 | -0.02 |
| 2003 Camp shallow | | | | | -0.06 | 0.00 | 0.00 | 0.01 | -0.01 | **0.03** | 0.01 | 0.01 | 0.02 | 0.01 | 0.00 | **0.03** | 0.00 | **0.03** | 0.01 |
| 2003 Camp mid-water | | | | | | -0.06 | -0.07 | -0.05 | -0.02 | -0.04 | -0.06 | -0.07 | -0.06 | -0.05 | -0.07 | 0.00 | -0.06 | -0.04 | -0.07 |
| 2003 East side shallow | | | | | | | -0.01 | 0.01 | -0.01 | 0.02 | 0.00 | -0.01 | -0.01 | -0.01 | -0.01 | 0.02 | -0.01 | **0.02** | 0.00 |
| 2003 East side mid-water | | | | | | | | -0.03 | -0.02 | 0.00 | -0.04 | -0.03 | 0.00 | -0.03 | -0.02 | -0.03 | -0.02 | -0.02 | -0.01 |
| 2003 West Bay shallow | | | | | | | | | 0.01 | 0.01 | -0.01 | 0.01 | **0.02** | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| 2003 West Bay mid-water | | | | | | | | | | 0.03 | 0.00 | 0.00 | 0.01 | 0.00 | -0.01 | 0.00 | 0.00 | 0.03 | 0.02 |
| 2007 1st fence shallow | | | | | | | | | | | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | **0.04** | 0.00 | 0.00 | 0.02 |
| 2007 1st fence mid-water | | | | | | | | | | | | 0.00 | 0.00 | 0.00 | -0.01 | 0.01 | -0.01 | -0.01 | 0.00 |
| 2007 2nd fence shallow | | | | | | | | | | | | | 0.01 | 0.00 | -0.01 | 0.01 | -0.01 | 0.01 | 0.00 |
| 2007 2nd fence mid-water | | | | | | | | | | | | | | 0.00 | -0.01 | 0.01 | -0.01 | 0.01 | 0.00 |
| 2007 Camp shallow | | | | | | | | | | | | | | | -0.01 | 0.01 | -0.01 | 0.01 | 0.00 |
| 2007 Camp mid-water | | | | | | | | | | | | | | | | 0.02 | -0.01 | 0.01 | -0.01 |
| 2007 East side shallow | | | | | | | | | | | | | | | | | **0.02** | **0.02** | **0.03** |
| 2007 East side mid-water | | | | | | | | | | | | | | | | | | 0.00 | -0.01 |
| 2007 West Bay shallow | | | | | | | | | | | | | | | | | | | 0.00 |

* P< 0.05 after sequential Bonferroni correction

Table S6. Information on samples screened for parasites: number of individuals (N ind.), proportion of males and proportions of individuals infected with any trematode species (% infected), *Microphallus livelyei* (% mic), *Notocotylus gippyensis* (% m2), *Telogaster opisthorchis* (% tel) and *Apatemon* sp. (% f1).

| year | habitat | site | N ind. | % males | % infected | % mic | % m2 | % tel | % f1 |
|---|---|---|---|---|---|---|---|---|---|
| 2003 | shallow | 1st fence | 202 | 9.9 | 46.5 | 43.6 | 1.5 | 0.0 | 1.0 |
| 2003 | shallow | 2nd fence | 100 | 10.0 | 29.0 | 27.0 | 1.0 | 1.0 | 1.0 |
| 2003 | shallow | Camp | 108 | 24.1 | 36.1 | 32.4 | 2.8 | 0.9 | 0.0 |
| 2003 | shallow | East side | 103 | 24.3 | 10.7 | 5.8 | 2.9 | 1.0 | 1.0 |
| 2003 | shallow | West bay | 106 | 17.0 | 16.0 | 13.2 | 2.8 | 0.0 | 0.0 |
| 2003 | mid-water | 1st fence | 100 | 14.0 | 17.0 | 5.0 | 2.0 | 2.0 | 7.0 |
| 2003 | mid-water | 2nd fence | 100 | 7.0 | 7.0 | 2.0 | 0.0 | 3.0 | 2.0 |
| 2003 | mid-water | Camp | 89 | 6.7 | 7.9 | 4.5 | 0.0 | 0.0 | 1.1 |
| 2003 | mid-water | East side | 100 | 4.0 | 6.0 | 0.0 | 0.0 | 2.0 | 4.0 |
| 2003 | mid-water | West bay | 100 | 18.0 | 8.0 | 1.0 | 2.0 | 2.0 | 1.0 |
| 2003 | deep | 1st fence | 106 | 3.8 | 8.5 | 1.9 | 0.0 | 0.9 | 2.8 |
| 2003 | deep | 2nd fence | 100 | 2.0 | 12.0 | 0.0 | 0.0 | 3.0 | 4.0 |
| 2003 | deep | Camp | 100 | 1.0 | 6.0 | 3.0 | 1.0 | 0.0 | 2.0 |
| 2003 | deep | East side | 100 | 5.0 | 11.0 | 2.0 | 0.0 | 1.0 | 6.0 |
| 2003 | deep | West bay | 100 | 10.0 | 8.0 | 2.0 | 0.0 | 1.0 | 4.0 |
| 2007 | shallow | 1st fence | 100 | 21.0 | 40.0 | 35.0 | 3.0 | 1.0 | 1.0 |
| 2007 | shallow | 2nd fence | 100 | 24.0 | 32.0 | 21.0 | 9.0 | 1.0 | 0.0 |
| 2007 | shallow | Camp | 100 | 19.0 | 27.0 | 23.0 | 4.0 | 0.0 | 0.0 |
| 2007 | shallow | East side | 100 | 10.0 | 17.0 | 12.0 | 5.0 | 0.0 | 0.0 |
| 2007 | shallow | West bay | 100 | 24.0 | 7.0 | 2.0 | 4.0 | 0.0 | 1.0 |
| 2007 | mid-water | 1st fence | 100 | 20.0 | 18.0 | 11.0 | 0.0 | 2.0 | 6.0 |
| 2007 | mid-water | 2nd fence | 100 | 15.0 | 6.0 | 6.0 | 0.0 | 0.0 | 0.0 |
| 2007 | mid-water | Camp | 100 | 22.0 | 9.0 | 4.0 | 0.0 | 1.0 | 4.0 |
| 2007 | mid-water | East side | 101 | 10.9 | 7.9 | 1.0 | 2.0 | 1.0 | 1.0 |
| 2007 | mid-water | West bay | 100 | 13.0 | 25.0 | 15.0 | 1.0 | 4.0 | 4.0 |
| 2007 | deep | 1st fence | 100 | 2.0 | 7.0 | 2.0 | 0.0 | 3.0 | 1.0 |
| 2007 | deep | 2nd fence | 103 | 4.9 | 4.9 | 1.0 | 1.0 | 0.0 | 2.9 |
| 2007 | deep | Camp | 100 | 5.0 | 12.0 | 7.0 | 0.0 | 2.0 | 3.0 |
| 2007 | deep | East side | 100 | 5.0 | 11.0 | 3.0 | 0.0 | 5.0 | 2.0 |
| 2007 | deep | West bay | 98 | 3.1 | 14.3 | 5.1 | 0.0 | 1.0 | 5.1 |

# Chapter IV

## Fitness distribution of asexual lineages in a natural population of coexisting sexuals and asexuals

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**Abstract**

Theory predicts that frequency-dependent selection by parasites which is expected to favour genetically diverse sexual populations, would also select for a diverse assemblage of clones, provided that there is high clonal diversity and the asexuals have the expected reproductive advantage over sexuals. In the population of freshwater snails *Potamopyrgus antipodarum* in Lake Alexandrina (New Zealand) sexual individuals co-exist with a genetically-diverse asexual clones. This poses a challenge to our current understanding of the processes underlying coexistence of sexual and asexual lineages and the maintenance of sex. Previous research has established the Red Queen hypothesis as a possible explanation for the maintenance of sex in this snail and shown that coexistence of sexuals and clones in Lake Alexandrina is stable, at least over 20 years.

To asses the extent to which the asexual lineages realize the predicted the two fold reproductive advantage we measured the reproductive output of sexual and asexual lineages in this natural lake population using experimental enclosures. We found that the reproductive output of asexual lineages is as high as that of the best sexual families, which implies that sexuals pay the cost of sex because sexuals also produce males. We also found that ranking of the asexual lineages by their reproductive output depends on the habitat they experience, which implies that environmental heterogeneity may select for habitat-specific clonal assemblages and promote maintenance of clonal diversity in the lake.

**Introduction**

The maintenance of sexual reproduction in nature calls for an evolutionary explanation of its short-term advantages. Assuming that sexual and asexual females produce the same number of offspring, asexual females would quickly replace the sexual population, because their intrinsic growth rate is twice as high as the sexual growth rate, as they do not produce males (Maynard Smith 1978). This theoretical two-fold advantage of asexuals is unlikely to be exactly accurate, because the extent of the asexual advantage depends on other factors which can influence the outcome of competition between sexual and asexual lineages. These factors include both further disadvantages of sexual reproduction, which make the potential cost of sexual reproduction higher than two fold (reviewed in: Lewis 1987; Meirmans et al. 2012) and also the disadvantages or constraints of asexual reproduction which can reduce the baseline two-fold cost. Further disadvantages to sex include be the time and energy invested in mate searching, courtship and copulation and risks associated with these activities, for example, sexually transmitted diseases or selfish genetic elements, or the cost of recombination (breaking up advantageous associations between genes) (Crow 1994, 1999). On the other hand, other factors can reduce the cost of sex or even completely reverse the cost, to benefit sexual reproduction. First, if developmental or genetic constraints on the evolution of asexuality exist, it makes the cost of sex inapplicable, e.g. in mammals (Engelstadter 2008). Other factors that can mitigate the cost of sex include female-biased sex ratio and paternal care. Further factors include ecological differentiation between sexuals and asexuals, which can reduce the competition between them (Schenck and Vrijenhoek 1986; Amat et al. 2006; Lehto and Haag 2010) and result in reducing the cost of sex.

A possible source of phenotypic differentiation between sexuals and asexuals is elevation of ploidy level, which is common in asexuals (Suomalainen 1987; Otto and Whitton 2000). Polyploidy is known to affect many important traits, from cell metabolism to body size to gene expression, and is also implicated cause for epigenetic instability and a high frequency of aneuploidy (Adams and Wendel 2005; Comai 2005), which in turn can influence the fitness-related traits. Given the complexity of factors affecting fitness of both sexual and asexual individuals, the assessment of the reproductive advantage of asexuals should involve a direct estimate of fitness. Usually measuring fitness of animals in a near-natural setting poses a challenge for empirical methods, therefore it is not surprising that studies reporting

fitness of coexisting sexual and asexual lineages are not numerous (but see e.g.: Lehto and Haag 2010).

Theoretically, a single asexual lineage possessing a reproductive advantage over sexuals is able to replace the sexual population (Lively 1996; Jokela et al. 1997a). The presence of multiple asexual lineages has important implications for the maintenace of sex in a population with mixed reproductive modes, as the hypotheses that invoke generation of diversity as the explanation for the maintenance of sex start to suffer. For example, the main difficulty for the Red Queen hypothesis to provide a full explanation for the maintenance of sex is that it selects for *rare* genotypes independently of the kind of process (sexual or asexual reproduction) that generates them (Lively and Howard 1994). When asexual population is as genetically diverse as the sexual one, parasite pressure is not enough to account for the two-fold cost of sex and sexual lineages are expected to go extinct (Howard and Lively 1994; Lively and Howard 1994; Lythgoe 2000). This has been formally shown by a mathematical model (Lively and Howard 1994): frequency-dependent selection exerted by parasites may select for clonal diversity as well as for sex. It has also been shown that asexual populations under strong parasite pressure are more diverse than populations with low parasite pressure (King et al. 2011). As long as asexuals have a reproductive advantage, a diverse set of clones can replace a sexual population (Lively and Howard 1994).

In the light of this model, the presence of extremely diverse set of asexual lineages co-existing with sexual population poses a challenge for our current understanding. However, if there is variation in fitness among different asexual lineages, such that not all of the asexuals are equally able to compete with the sexuals, the apparent diversity among asexual lineages is functionally lower. In other words, one would like to know what is the diversity of asexual lineages that can put sexuals under pressure.

In this study we aimed to assess the variation in fitness among asexual lineages of a freshwater snail *Potamopyrgus antipodarum* in a well-studied lake population in Lake Alexandrina (New Zealand, South Island). This population consists of a very diverse set of asexual lineages which co-exists with a sexual population (Fox et al. 1996; Chapter III). Sexual *P. antipodarum* are more common in the shallow part of the lake, where the risk of infection with the castrating trematode parasites is also highest (Jokela and Lively 1995).

Each of the the depth-related habitat zones harbour a unique set of clones, and only a few clones are shared between the habitats (Fox et al. 1996; Chapter III).

In a previous study, the cost of sex has been assessed in *P. antipodarum* by measuring several life history traits of sexual and asexual individuals collected from a single natural population and by conducting a laboratory-based competition experiment between an asexual clone and a sexual population (Jokela et al. 1997a). Jokela et al. (1997a; 1997b) found that key life history and reproductive traits were similar between coexisting sexual and asexual in each of the three habitats that were sampled. Relative growth rate of the asexual clone was higher than that of the sexuals in the competition experiment. Taken together, these results indicate that there is a cost of sex for *P. antipodarum*, at least in the context of the clones sampled in these experiments (Jokela et al. 1997a; Jokela et al. 1997b). More recent studies, however, suggest that the all-else-equal assumption may not hold for *P. antipodarum* clones. Jokela et al. (2003) showed that clonal lineages from the same lake population varied dramatically in their reproductive success. They also found that many lineages did not reproduce quickly enough to replace themselves. However, this experiment was performed in laboratory conditions, and it is hard to tell how relevant the fitness estimates obtained in the lab reflect the actual fitness of these lineages in the natural conditions.

The discrepancy between the results of the above mentioned studies was a motivation to carry out a field experiment aimed at measuring fitness of asexual lineages of *P. antipodarum* in their natural environment. We used experimental enclosures (cages anchored to the bottom of the lake) to measure reproductive output of single snails from the mid-water habitat over the course of one year. We predicted that, if the variation in fitness among the clones is as high as observed in the study by Jokela et al. (2003), and many lineages have low fitness, the number of asexual lineages which are potentially able to outcompete sexuals is lower than predicted from observed genotypic diversity among them. If only a few asexual lineages have high fitness, the persistence of the sexual population is easier to explain by parasite-mediated selection focusing on few common clones. Because we were constrained by the number of cages we could handle in total, we were able to focus on one habitat only, to make sure that we obtained replicate lineages of common clones in the random sample enclosed in the cages. We decided to focus on the mid-water habitat, because the infection rates there are lower than in the shallow (Jokela and Lively 1995), so the risk that many individuals would not

reproduce in cages due to infection is lower. By doing so, we still include asexual lineages that in nature directly compete with their sexual counterparts, as the the proportion of sexuals in mid-water habitat is 10-50 % (and varies in time) (Fox et al. 1996; Chapter III).
The genetic structure of the asexual population in lake Alexandrina is habitat-related, that is, each of the depth-related habitats harbours a distinct set of clones (Fox et al. 1996; Chapter III). In the second part of this study, our aim was to unravel the factors responsible for maintaining this structure. A previous study (Negovetic and Jokela 2001) revealed that survival of asexual snails may be poorer in foreign habitas. We hypothesized that if there is a strong local adaptation of asexual lineages to their native habitat, clones would have a lower fitness in a "foreign" habitat, and highest fitness in their "home" habitat. We tested this hypothesis by measuring reproductive output of descendants of iso-female lineages obtained in the first part of the experiment. We used experimental cages to split the families to all three littoral habitats simultaneously for one year. We expected that clones would have highest fitness in their home habitat (mid-water) and lower in the foreign habitats (shallow and deep). We also tested for the possiblity that fitness rank of clones is habitat-specific selecting for different top clones in different environments. If the advantage of clones is limited to a certain habitat zone, such result would also imply that the overlap of ecological niches of sexual and asexual lineages is narrow, and broadens the conditions for maintenance of sex.

## Methods

*Study system*

*Potamopyrgus antipodarum* is a prosobranch snail, native to New Zealand freshwater lakes and streams, where obligately sexual individuals often coexist with obligately asexual individuals in variable proportions (Winterbourn 1970; Lively 1987; Wallace 1992; Dybdahl and Lively 1995; Neiman et al. 2011).
Lake Alexandrina is a mesotrophic sub-alpine lake (Ward and Talbot 1984) on the South Island of New Zealand. There are three major habitat zones: (i) shallow (<0.5 m, near the shore bank, defined by willow roots), (ii) mid-water (1-3 m, dominated by the macrophyte *Isoetes alpinus*) and (iii) deep (4-6 m, dominated by the macrophytes *Elodea canadensis*, *Myriophyllum triphyllum* and *Chara* sp.) (Ward & Talbot, 1984). Sexual (diploid) and asexual

(triploid) snails coexist in this lake, and their relative proportions vary according to depth-related habitats, with sexuals most common in the shallow and asexuals dominationg the deep (Fox et al. 1996; Chapter III). Clonal diversity among asexual lineages is very high, and each of the habitats harbours a distinct assemblage of clones (Fox et al. 1996; Chapter III).

*Field experiments*

We collected a random sample of *P.antipodarum* from the mid-water habitat in Lake Alexandrina, New Zealand (between sites called "Camp" and "SW-End" in Jokela and Lively (1995) in January 2010. We discarded the snails smaller than 2 mm through sieving in order to retrieve sexually mature individuals. Although this threshold did not assure that all included snails were sexually mature, we did not use a larger threshold in order not to exclude any inherently small-sized lineages. From this random sample, 200 individuals were transported to Edward Percival Field Station in Kaikoura and frozen in liquid nitrogen for genotyping, and 521 individuals were distributed into field cages on the same day. We used modified Jokela cages, which consist of a plastic (PET) bottle frame (250 ml) with four cut-out holes on the sides. Each of the holes was approximately 9x4 cm large. Around the bottle frame a mesh sleeve (250 µm opening) was attached tightly using cable binders around the bottle neck and at the bottom of the loose mesh (Figure 1). We used 250 µm mesh size to ensure all newborn babies stay inside the cage. The experimental (N=521) and control (N=5) cages were tied together on ropes (24 to 27 cages per rope) and the 21 ropes were anchored to the lake bottom at Site 1 (100 m north of the site called "West Bay" in Jokela and Lively (1995), in the mid-water habitat zone (depth of 1.5 to 2m) using gravel bags and stones. Together with a snail we put into each a cage a piece (approx. 7 cm long) of thoroughly washed *Elodea canadensis* plant to provide an initial food source before the periphyton colonizes the surfaces of the cage. As a control for presence of small juvenile *P.antipodarum* on *Elodea* plants, we used 5 cages with only *Elodea canadensis.*

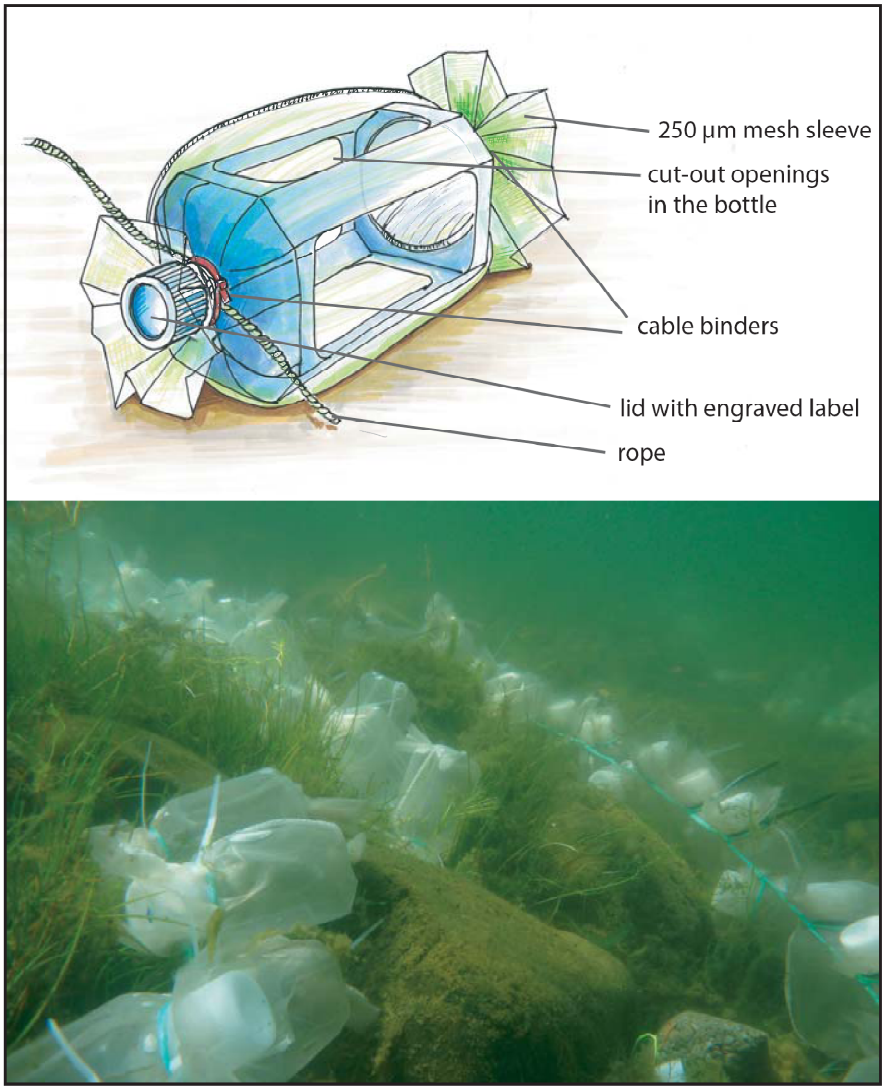


Figure 1. Schematic drawing of a 250ml Jokela cage and an underwater photograph of cages anchored to the bottom of Lake Alexandrina.

The snails were left in cages in the lake for one year and taken out within 11 subsequent days in January 2011.

Upon retrieval, each cage was visually inspected for holes or tears in the mesh. In case the mesh was damaged, the cage was discarded. Intact cages were first washed on the outside in order to remove any possible snails that may be crawling on them, and then cut open in a tray filled with clean lake water. All snails in one cage were packed into a wet paper towel and transported to Mt John University Observatory in Lake Tekapo where each family was

photographed on white background, together with a millimeter-scale ruler using a digital camera for subsequent analysis. One to three individuals (if present) from each family were then frozen in liquid nitrogen for genotyping. All families which initially had 18 or more individuals were kept alive (N=123) and 46 of these were randomly chosen for the follow-up transplant experiment.

From each of these 46 families that were chosen for transplant experiment we randomly chose 15 individual snails. These individuals were distributed into a new set of cages, one snail per cage. We used cages of the same kind as described above, similarly tied into ropes (25-27 cages per rope). We placed 10 ropes in each of the depth-specific littoral habitats (shallow, mid-water, and deep). In each habitat, each 5 individuals from each family were distributed into a cage attached to a different rope. As before, we put a piece of *Elodea canadensis* plant to each cage as an initial food source for the snail. We used 66 cages as controls. We put a piece of *E. canadensis* as a control for presence of small juvenile *P.antipodarum* on *Elodea* plants (36 cages), and left 30 cages completely empty, to additionally control for imprecision of the cage manufacturing, i.e. to check if any snails from the outside are able to get in. For both "*Elodea*-controls" and "empty-controls", we placed at least 10 cages across at least 5 ropes in each habitats.

Within 5 subsequent days in January 2012 all cages were retrieved and the content photographed, following the protocol described for 2011 (see above). One to two snails (if available) from each family were frozen in liquid nitrogen for genotyping. If the remaining number of snails was more than 9 individuals, they were transported alive to Edward Percival Field Station in Kaikoura and maintained in culture for future experiments.

We analyzed all photos using ImageJ software (Rasband 1997-2012), using the function "Analyze Particles" with images converted to binary (black snails on white background). For each image, we recorded the count of snails.

*Genotyping*

Two individuals (or one if only one was present) from each family recovered both in 2011 and in 2012, as well as 200 individuals from the random sample collected in 2010 were genotyped using 9 polymorphic allozyme loci (6PGD, PEP-D, MPI, IDH1, IDH2, AAT1, AAT2, PGM1, PGM2) using cellulose acetate electrophoresis. We followed the protocol

described in detail in Fox et al. (1996) and Jokela et al. (2009). For identification of the clonal genotypes, we used the same method as described in Jokela et al. (2009): we identified diploid individuals by the presence of two alleles at at least one locus, and diploids were distinguished from triploid heterozygotes by the presence of asymmetric banding patterns in the latter.

*Statistical analysis*

We used fecundity and individual size as the performance measures. Fecundity estimates were based on the count of snails found in the cages after one year in the lake. We made no effort to identify the original female as the offspring were already adult when the cages were retrieved. Some offspring had reproduced; therefore, for all analyses of fecundity we excluded juveniles that were smaller than 1.8 mm. These individuals were likely to be second generation (F2) offspring of the reproductively mature offspring of the female that was isolated in 2010.

In the first year experiment we analyzed fecundity either using generalized linear mixed models (GLMMs, with negative binomial error distribution and log link function), or using generalized linear models (GLMs, after ln-transformation of fecundity counts). When we compared the fecundity of sexual lineages, common clones and rare clones we corrected for the effects of rope at which the cage was attached to by allowing each lineage type have a rope specific mean. We did this by fitting a random factor "lineage type × rope" (including 62 random effects). In general, type of lineage (sexual, common clone, rare clone), or genotype identity, were used as a fixed factors in separate models. Due to overdispersed data we achieved better model fits when we used a negative binomial distribution with log link function than when using a Poisson distribution. In the analysis including the 16 best performing sexual lineages the performance of lineages was corrected for the effect of rope in which each cage housing the lineage was attached to by including rope as a random effect in the model. In the analyses comparing performance of 11 common clones the variance due to rope at which the cages were attached to was small and not included in the statistical model.

We analyzed the fecundity of lineages belonging to the 11 common clones in the second year experiment with a GLLM where habitat, commonness of the clone (common, rare) and the genotype of the clone (nested under commoness) were treated as fixed factors. It is unusual to use a nested fixed factor, but in our case this is justified because we were interested in specific differences in fecundity among clones and how those relate to differences in their frequency. Additionally, we were also specifically interested in estimating the habitat × clone interaction to assess if performance of clones was habitat specific (see also: Siemer and Joormann 2003). We included rope and lineage identity as random factors in the model. Lineage refers to replication of common genotypes in cages held in the lake for the year 2011. Each of these lineages was replicated to three habitats for year 2012 as described above. An unfortunate complication in the analysis was the loss of many cages in the shallow habitat, which introduced unbalance in the data. More specifically, for some of the common genotypes we only have fecundity estimates from the mid-water and deep habitats.

**Results**

We retrieved 80 % (417) of the original 521 experimental cages and 5 (100%) of 5 control cages intact. The losses were due to abrasion-related holes in the mesh and not specific to any of the 21 ropes in which the cages were attached to. We found snails in 412 (98%) of the intact cages. All together we counted and measured 10624 snails, out of which 7133 were above 1.8 mm. Of the five control cages, which were initiated with the food plant, but otherwise empty, three had no snails, one had 3 snails and one had one snail (mean ± SE = 0.80 ± 0.58 snails). This indicates that some snails invaded either as attached to the initial food plant, or as exceptionally small new-born (mesh size was 250 microns). As the mean number of invaders per cage was low and the invaders had to be very small at the point of invasion, and thus non-reproductive, we are confident that the fecundity estimates for the enclosed adult females (mean ± SE = 17.11±1.06) are not significantly affected by possible invaders. Five cages returned completely empty (1.2%) and 47 with one snail only (11.3%), which indicates that survival of snails in the cages was very high during the experiment. Two thirds of the cages had more than three snails after the 12 months in the enclosure, indicating that most snails reproduced in the cages.

Genotyping of the material left 99 of the 412 families unresolved with respect to genotype or ploidy level. Among the remaining resolved families we found 162 diploid sexual and 151 triploid asexual families. We divided the triploid families to 104 families representing common clones and 47 families representing rare clones. Assignment to common and rare clones was based on the random sample of 200 genotyped individuals from the same collection that was initially used to populate the cages. We assigned a clone as common if it represented at least 1.5% of the sample, which resulted in 11 common clones. In the cage experiment, each common clone was found replicated by at least three independent families. Number of families per genotype found in the experiment correlated positively with the frequency of that genotype in the background sample (Pearson r = 0.94, n = 11, $P < 0.001$), as expected if assignment of snails to cages was random. The two most common clones were replicated to over 20 cages each.

Average number of offspring did not differ among common and rare clones (mean ± SE: 30.24 ± 4.12 vs 29.75 ± 5.17, pairwise contrast: p = 0.941), but diploid families were statistically significantly smaller than either of the clonal groups (mean ± SE: 5.04 ± 0.64; pairwise contrasts p < 0.001) (Table 1).

Table 1. Generalized linear mixed model results showing the variation in fecundity in first year of the experiment (2011) explained by lineage type (common clone, rare clone, all sexuals). See Methods for details.

| Model: fecundity ~ lineage type + type × rope | | | AIC =983, BIC = 987 | |
|---|---|---|---|---|
| Prob dist: Neg.Bin, Link function: Log | | | | |
| **Fixed effects** | **F** | **Df1** | **Df2** | **p-value** |
| Type of lineage | 58,0 | 2 | 77 | <0.001 |
| **Random effects** | **Variance estimate** | **SE** | **Z** | **p-value** |
| Type × Rope | 0.187 | 0.066 | 2.827 | 0.005 |

Although *Potamopyrgus* snails, alike to many other gastropods, are reported to store sperm for many months (Wallace 1992), it is possible that some sexual females experienced sperm limitation. Therefore, we examined the fecundity of the most productive 10% (N=16) of the

sexual families separately. As these 10% produced 60% of all diploid offspring we considered that each of these families represents a good comparison to the best performing clonal lineages and would represent the group that asexuals need to outcompete in order to increase in frequency in mixed populations. Comparison of common clones to these best performing sexuals also reveals if sexuals can compensate for the cost of males. We found that the average number of offspring produced by the best performing sexuals was 16% higher than that of the common clones, but the difference was not statistically significant due to large variance in reproductive output (lineage type, $F_{(2, 144)} = 0.442$, $P = 0.644$). Nevertheless, the result suggests that when male production is discounted (1:1 sex-ratio), the best sexual lineages have lower growth rate than the best asexual competitors (Figure 2). In other words, it appears that the sexual lineages pay the cost of males.

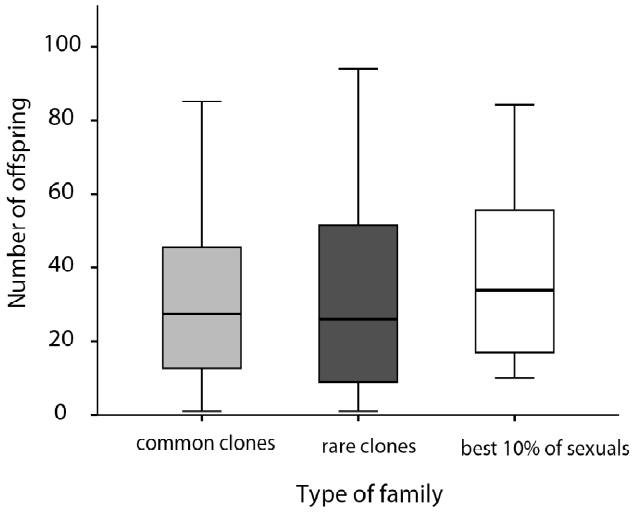


Figure 2. Boxplot showing median and quartiles of the number of offspring produced after 1 year by common clones, rare clones and 10% of sexual families with the highest reproductive output.

We examined the genetic basis of variation in fecundity by analyzing the within-genotype and among-genotype variance components in fecundity in these common clones. We found that 13% of the variance in fecundity of the families in the experiment was explained by the genotype ($F_{10, 93} = 2.03$, MS = 0.122, p=0.039, Figure 3). Fecundity of the genotypes did not correlate with their frequency in the random sample (Pearson r = -0.17, N = 11, p=0.62).

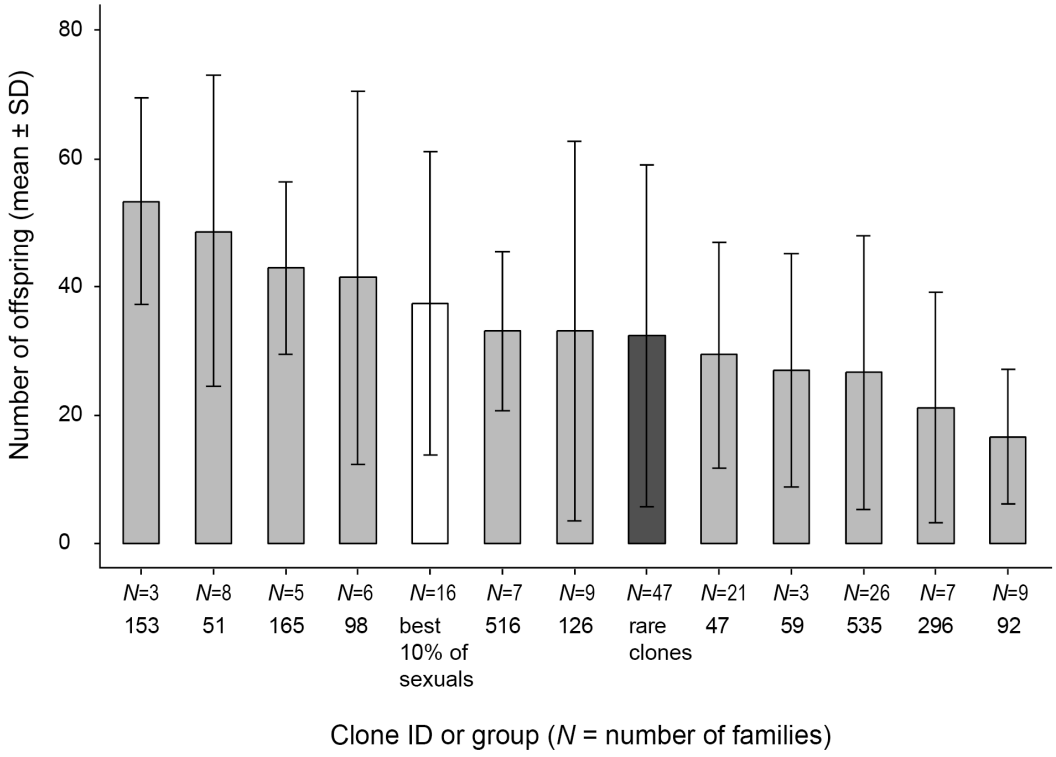


Figure 3. Mean number of offspring produced by the common clones (light grey), rare clones (dark grey) and the best 10% of the sexuals (white). For common clones the genotypes for which at least three replicates with the same genotype were retrieved in 2011 are included. *N* denotes the number of families. Error bars represent standard deviation. See Results for the criteria of assignment into common or rare category

In 2011 we installed 688 experimental cages each with a single snail to continue the lineages of the first year for one more year. For this purpose we chose 46 families, which were a random sample of the 123 largest families (each with more than 18 offspring). Each of these families was split to five cages each in the three littoral habitat zones. We lost 79%, 15% and 1% of the cages installed in shallow, mid-water and deep habitats, respectively. As in the previous year, the losses were due to abrasion-caused holes in the mesh. High losses in the shallow habitat were to be expected because heavy wave action and storms are frequent in Lake Alexandrina. This left us with 47, 195 and 228 intact cages in shallow, mid-water and deep habitats, respectively. Only 26 cages (5.5 %) returned completely empty of snails.
In 2011 we also included 36 empty control cages with the food plant (13, 10 and 13 cages in shallow, mid-water and deep, respectively) and 30 control cages that were completely empty

(10 in each habitat). Of these controls two shallow, 12 mid-water and 23 deep cages returned intact. One third of the control cages with the food plant were empty (N=7), 38% had one snail (N=8) and two cages had more than one individual. Of the control cages that were started empty 56% remained empty to the end of the experiment. Four of the "empty" control cages had more than three snails (25%). The average number of snails found in the control cages was roughly tenth of the average found in the experimental cages (mean ± SE: 2.4 ± 1.3 and 1.6 ± 0.55 snails per cage for controls with and without food plant respectively, 21.54± 1.1 snails per cage for experimental cages).

In the end of the second year of the experiment, 444 cages contained at least 1 snail that was larger than 1.8 mm. Among these, we excluded all cages from the statistical analyses where the initial lineage (i.e. family) had an unresolved genotype or the two genotyped snails in 2012 had mismatching genotypes, or genotypes of snails between the parent family of 2011 and offspring of 2012 did not match. Six of the initial families were diploids, but the 32 diploid cages that we retrieved could not be included in the following analyses because of unproportionally high losses in the shallow habitat. After excluding these families, 20 (44%), 98 (51%), 109 (52%) cages for shallow, mid-water and deep habitats respectively remained for the analysis. The 33 initial asexual families represented 16 genotypes, including 10 out of 11 clones that we identified as common in the random sample of mid-water habitat (Table 3). Losses of cages due to holes and losses due to non-matching genotypes led to unbalanced replication, especially in the shallow habitat.

During the second year of the experiment average fecundity was highest in the mid-water habitat, followed by shallow and deep (mean ± SE: 30.8 ± 4.0, 25.2 ± 6.1 and 18.1 ± 2.5, respectively). Main effect of habitat on fecundity was statistically significant in the GLLM (Table 2). Pairwise contrasts revealed that this was due to a significant difference in fecundity between mid-water habitat and deep habitat. Althouh the mean fecundity in the shallow habitat was nearly as high as in the mid-water habitat, the statistical power to detect significant difference was lower due to unbalanced data. We also found statistically significant interaction between clone and habitat, suggesting that the clones differed in their habitat specific fecundity (Figure 4), although the average differences in performance of the clones were not significant (main effect of genotype in Table 2). Rare clones had 15% lower fecundity than the common clones (21.4 ± 2.7 vs. 25.3 ± 4.1, for common and rare clones

respectively, Figure 5) but the difference was not statistically significant (Table 2) Partly the apparently weak genotype effects may be due to low level of replication, which leads to low statistical power, but it may also be because we chose initially high-performing clones to continue the experiment for the second year.

Table 2. Generalized linear mixed model results showing the variation in fecundity in the second year of the experiment (2012) explained by clone type (common, rare), habitat (shallow, mid-water, deep), and genotype. See Methods for details.

| Model: fecundity ~ habitat + clonetype + genotype(clonetype) + habitat × clonetype + habitat × genotype(clonetype) + lineage + rope(habitat)<br>Prob dist: Neg.Bin, Link function: Log | | | AIC =491, BIC = 497 | |
|---|---|---|---|---|
| **Fixed effects** | **F** | **Df1** | **Df2** | **p-value** |
| Habitat | 3.92 | 2 | 127 | 0.022 |
| Clonetype | 0.16 | 1 | 83 | 0.693 |
| Genotype(clonetype) | 1.74 | 14 | 26 | 0.108 |
| habitat × clonetype | 0.94 | 2 | 184 | 0.391 |
| habitat × genotype(clonetype) | 1.91 | 23 | 184 | 0.010 |
| **Random effects** | **Variance estimate** | **SE** | **Z** | **p-value** |
| Lineage | 0.022 | 0.045 | 0.482 | 0.630 |
| Rope(habitat) | 0.025 | 0.032 | 0.482 | 0.445 |

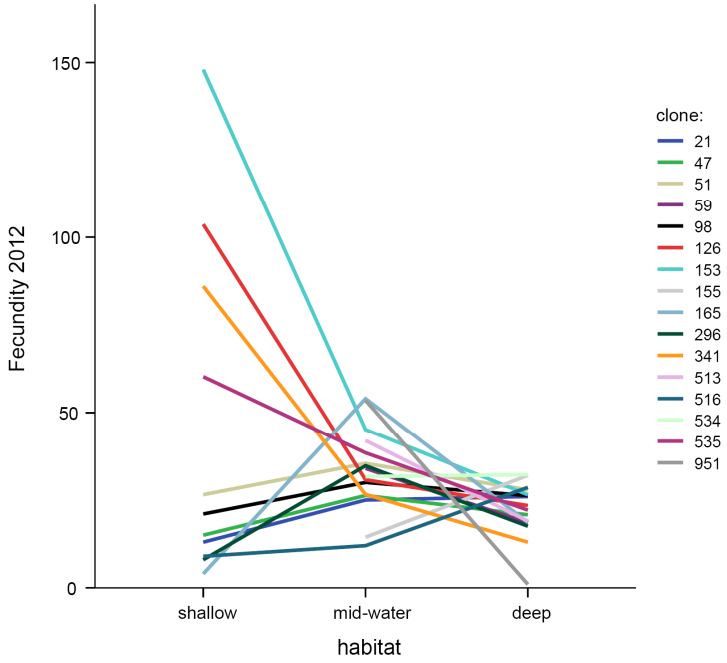


Figure 4. Mean fecundity of the clonal lineages in the three habitats in the second year of the experiment (2012).

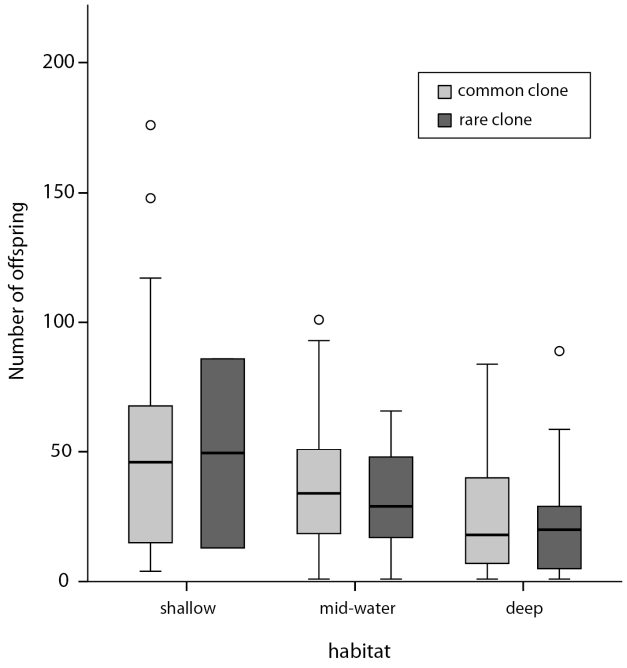


Figure 5. Boxplot showing median (with quartiles) of the number of offspring produced in the three habitats (shallow, mid water and deep) by common (light grey) and rare clones (dark grey).

Table 3. Numbers of replicate families within the different clones used the in the second year experiment.

| clone | families retreived 2011 | families starting second year | families retrieved end of second year | | | |
|---|---|---|---|---|---|---|
| | | | shallow | mid-water | deep | Sum second year |
| 535* | 26 | 6 | 7 | 15 | 18 | **40** |
| 47* | 21 | 4 | 1 | 10 | 9 | **20** |
| 126* | 9 | 2 | 3 | 8 | 7 | **18** |
| 51* | 8 | 2 | 2 | 9 | 10 | **21** |
| 296* | 7 | 4 | 1 | 12 | 13 | **26** |
| 516* | 7 | 2 | 1 | 3 | 7 | **11** |
| 98* | 6 | 1 | 1 | 4 | 4 | **9** |
| 165* | 5 | 2 | 1 | 5 | 9 | **15** |
| 59* | 3 | 1 | 0 | 1 | 2 | **3** |
| 153* | 3 | 3 | 1 | 12 | 12 | **25** |
| 21 | 2 | 1 | 1 | 1 | 4 | **6** |
| 155 | 2 | 1 | 0 | 5 | 4 | **9** |
| 341 | 2 | 1 | 1 | 5 | 4 | **10** |
| 513 | 1 | 1 | 0 | 1 | 1 | **2** |
| 534 | 1 | 1 | 0 | 3 | 4 | **7** |
| 951 | 1 | 1 | 0 | 4 | 1 | **5** |
| total | **104** | **33** | **20** | **98** | **109** | **227** |

*denotes the 10 out of 11 most common clones in the random sample of 2010

To evaluate how predictable fecundity of lineages was from one generation to the other, we compared the reproductive output of each lineage in 2012 to that in 2011. We split this analysis by habitat experienced in the second year to examine if heritability of performance was affected by the environment. Interestingly, the reproductive output of lineages in 2011 correlated with the average reproductive output of offspring lineages in 2012 in the mid-water habitat where the lineages originated from (Pearson r = 0.39, N=33, P=0.023), but not in the shallow (Pearson r=-0.23, N=15, P=0.406) or in the deep habitats (Pearson r=0.170, N=33, P=0.343) (Figure 6). This suggests that relative performance of lineages responds to environmental variation.

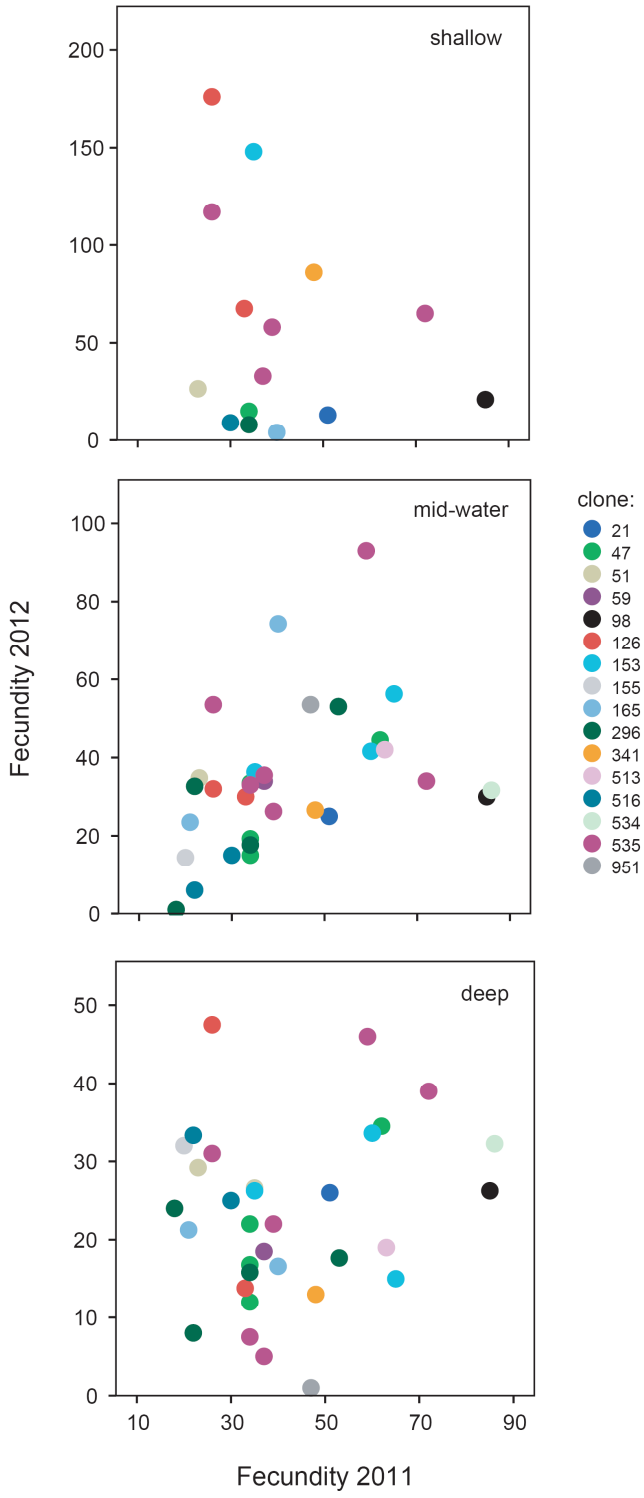


Figure 6. Comparison of fecundity of the asexual lineages between years 2011 and 2012 in the three habitats (shallow, mid-water and deep). Genotypes (clone numbers) are denoted by colours.

**Discussion**

The maintenance of sex in the freshwater snail *P.antipodarum* has been attributed to the frequency-dependent selection by parasites (Jokela et al. 2009), however the very high clonal diversity present among the asexuals poses a challenge to the full understanding of the processes responsible for maintaining sex and allowing the coexistence of sexual and asexual lineages. This is because the Red Queen hypothesis for the maintenance of sex is not expected to account for coexistence of sexual and asexual lineages and maintenance of sex when the clonal assemblages are diverse (Lively and Howard 1994). The prediction is that as long as the asexual lineages have a reproductive advantage over sexuals, a diverse set of clones can replace the sexual population.

Our first goal was to evaluate the fitness distribution of the clones under near natural conditions. This would reveal the diversity of clones that can successfully compete with the sexuals. It is a methodological challenge to obtain an estimate of fitness for clonal lineages in the natural conditions, but this it is a crucial prerequisite for assessing the cost of sex. We measured reproductive output of sexual and asexual females in the field experiment and found that the reproductive output of the asexual lineages was not significantly different than the reproductive output of the 10% best sexual lineages. This suggests that after accounting for the production of male offspring by sexual lineages, their growth rate is lower that the asexual lineages, confirming the results of the previous study there is cost of males in sexual *P. antipodarum* (Jokela et al. 1997a).

We expected that many of the rare clones will have low fitness, as had been observed in the study by Jokela et al. (2003), where in a laboratory-based study many of the asexual lineages did not reproduce quickly enough to replace themselves and many not reproduce at all. Instead, we did not find a significant difference in the reproductive output of common and rare clones (Figure 2). Also, the frequency of clones in the random sample did not correlate with the reproductive output measured in cages. For example, the two most common clones (535 and 47) did not have the highest reproductive output in our experiment. This suggests that there must be other important selective factors than intrinsic reproductive rate that contribute to the observed frequencies of the sexuals and asexuals in the wild. While measuring the reproductive output in cages, we exclude for example the effects of intraclonal

competition, predation and parasitism. In previous studies using population cages in Lake Alexandrina (J. Jokela, unpublished data) very low infection rates were observed (<2%). Infection rates in the field are much higher (Jokela and Lively 1995) and genotype specific (Jokela et al. 1997b; Lively 2000; Jokela et al. 2009). It has been demonstrated, that over the course of 7-10 years common clones have been replaced by initially rare clones and that initially resistant common clones became susceptible to the sympatric (but not allopatric) parasites (Jokela et al. 2009). The temporal changes in the clonal structure have also been demonstrated to be more rapid in the habitats where parasite pressure is high, but not in habitats where the parasite pressure is low (Chapter III). Both of these studies suggest that parasites are able to drive the common clones to lower frequency. Together with the results of this study, where we found that reproductive output of majority of clones is high enough to succesfully compete with the sexual population, it appears that without strong ecological interactions (e.g. parasitism, intraclonal competition) in natural populations the clones would have the capacity to replace the coexisting sexuals.

However the question still remains: why are the resistant clones from the deep habitat, which harbour an extremely diverse set of clones not replacing the sexuals in the shallower habitats? Because the clonal population is structured according to habitat in Lake Alexandrina, i.e. each habitat harbours a distinct set of clones (Fox et al. 1996; Chapter III), we expected that the clones may be locally adapted to their 'home habitat' and will have lower fitness in the 'foreign' habitats. We found significant differences among reproductive output of the clones (initially collected in the mid-water habitat) between habitats. Pairwise contrasts revealed that there was a significant difference in fecundity between mid-water habitat and deep habitat. Unfortunately the losses of cages in the shallow habitat due to abrasion were very high, so the estimate for this environment is also laden with the highest error. Interestingly, the analysis of genetic correlation (i.e. with clonal lineages) of the reproductive performance between the two year showed that there was a significant correlation in the reproductive performance in the mid-water habitat (where the clones originally came from) but no such correlation was found for shallow and deep. So even though we did not find a consistently lower performance in the foreign habitats, this suggests that there is fitness of the clones is to some degree environment specific.

A previous study by Negovetic and Jokela (2001) found in the same population of *P.antipodarum,* that asexual snails had higher survival in their native habitat than in a foreign habitat. The role of ecological differentiation in maintaining the coexistance between sexual and asexual forms has previously been demonstrated (e.g. Case 1990; Vorburger et al. 2003; Verduijn et al. 2004; Lehto and Haag 2010). At the moment we can only speculate what kind of environmental factors could be responsible for the observed pattern in our case. The habitats in Lake Alexandrina, being depth-related differ in light availability, which together with the differences in the macrophyte community likely differentiate also periphyton (which is the food source of the snails) in the different habitat zones. While abiotic and biotic environmental factors may be important determinants of success of specific genotypes, it would also be advantageous for future experiments to compare fitness of clonal lineages in natural populations under high population density and allowing competitive interactions among clonal lineages.

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**Literature cited:**

Adams, K. L., and J. F. Wendel. 2005. Novel patterns of gene expression in polyploid plants. Trends Genet. 21:539-543.

Amat, I., M. Castelo, E. Desouhant, and C. Bernstein. 2006. The influence of temperature and host availability on the host exploitation strategies of sexual and asexual parasitic wasps of the same species. Oecologia 148:153-161.

Case, T. J. 1990. Patterns of coexistence in sexual and asexual species of Cnemidophorus lizards. Oecologia 83:220-227.

Comai, L. 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet. 6:836-846.

Crow, J. F. 1994. Advantages of sexual reproduction. Dev. Genet. 15:205-213.

Crow, J. F. 1999. The omnipresent process of sex. J. Evol. Biol. 12:1023-1025.

Dybdahl, M. F., and C. M. Lively. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). J. Evol. Biol. 8:385-398.

Engelstadter, J. 2008. Constraints on the evolution of asexual reproduction. BioEssays 30:1138-1150.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50:1541-1548.

Howard, R., and C. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554-557.

Jokela, J., M. F. Dybdahl, and C. M. Lively. 2009. The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. Am. Nat. 174:S43-S53.

Jokela, J., and C. M. Lively. 1995. Spatial variation in infection by digenetic trematodes in a population of freshwater snails (*Potamopyrgus antipodarum*). Oecologia 103:509-517.

Jokela, J., C. M. Lively, M. F. Dybdahl, and J. A. Fox. 1997a. Evidence for a cost of sex in the freshwater snail *Potamopyrgus antipodarum*. Ecology 78:452-460.

Jokela, J., C. M. Lively, J. A. Fox, and M. F. Dybdahl. 1997b. Flat reaction norms and "frozen" phenotypic variation in clonal snails (*Potamopyrgus antipodarum*). Evolution 51:1120-1129.

King, K. C., J. Jokela, and C. M. Lively. 2011. Parasites, sex and clonal diversity in natural snail populations. Evolution 65:1474-1481.

Lehto, M. P., and C. R. Haag. 2010. Ecological differentiation between coexisting sexual and asexual strains of Daphnia pulex. J. Anim. Ecol. 79:1241-1250.

Lewis, W. M., Jr. 1987. The cost of sex. Pp. 33-57 *in* S. C. Stearns, ed. The evolution of sex and its consequences. Birkhäuser Verlag, Basel (Switzerland).

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519-521.

Lively, C. M. 1996. Host-parasite coevolution and sex - Do interactions between biological enemies maintain genetic variation and cross-fertilization? Bioscience 46:107-114.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405:679-681.

Lively, C. M., and R. S. Howard. 1994. Selection by parasites for clonal diversity and mixed mating. Philos. Trans. R. Soc. Lond. B Biol. Sci. 346:271-281.

Lythgoe, K. A. 2000. The coevolution of parasites with host-acquired immunity and the evolution of sex. Evolution 54:1142-1156.

Maynard Smith, J. 1978. The evolution of sex. Cambridge Univeristy Press, Cambridge.

Meirmans, S., P. G. Meirmans, and L. R. Kirkendall. 2012. The Costs Of Sex: Facing Real-world Complexities. The Quarterly Review of Biology 87:19-40.

Negovetic, S., and J. Jokela. 2001. Life-history variation, phenotypic plasticity and maintenance of subpopulation structure in a freshwater snail. Ecology 82:2804-2815.

Neiman, M., D. Paczesniak, D. M. Soper, A. T. Baldwin, and G. Hehman. 2011. Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. Evolution 65:3202-3216.

Otto, P., and J. Whitton. 2000. Polyploid incidence and evolution. Annu. Rev. Genet. 34:401-437.

Rasband, W. S. 1997-2012. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. http://www.imagej.nih.gov/ij/.

Schenck, R. A., and R. C. Vrijenhoek. 1986. Spatial and temporal factors affecting coexistence among sexual and clonal forms of Poeciliopsis. Evolution 40:1060-1070.

Siemer, M., and J. Joormann. 2003. Power and measures of effect size in analysis of variance with fixed versus random nested factors. Psychol. Methods 8:497-517.

Suomalainen, E. 1987. Polyploidy in association with parthenogenesis. Pp. 71-112 *in* E. Suomalainen, A. Saura, and J. Lokki, ed. Cytology and Evolution in Parthenogenesis. CRC Press, Boca Raton.

Verduijn, M. H., P. J. Van Dijk, and J. M. M. Van Damme. 2004. Distribution, phenology and demography of sympatric sexual and asexual dandelions (Taraxacum officinale s.l.): geographic parthenogenesis on a small scale. Biol. J. Linn. Soc. 82:205-218.

Vorburger, C., M. Lancaster, and P. Sunnucks. 2003. Environmentally related patterns of reproductive modes in the aphid Myzus persicae and the predominance of two 'superclones' in Victoria, Australia. Mol. Ecol. 12:3493-3504.

Wallace, C. 1992. Parthenogenesis, sex and chromosomes in *Potamopyrgus*. J. Molluscan Stud. 58:93-107.

Ward, J., and J. Talbot. 1984. Distribution of aquatic macrophytes in Lake Alexandrina, New Zealand. N. Z. J. Mar. Freshw. Res. 18:211-220.

Winterbourn, M. J. 1970. The New Zealand species of *Potamopyrgus* (Gastropoda: Hydrobiidae). Malacologia 10:283-321.

# Concluding remarks

The earliest explanation for the existence of sexual reproduction was that of Weissman (1904), who saw it as a mean of increasing variation in offspring and providing material on which natural selection can act. Fisher (1930) and Muller (1932, 1964) in concordance with Weissman's idea argued that sex is adaptive because it enhances the rate of adaptation. With the development of the evolutionary theory, in 1970's, it became apparent to Maynard Smith (1971, 1978) and Williams (1975) that the Fisher-Muller model 1) provides advantage on the time scale which is much longer than the timescale at which the cost of sex becomes apparent; 2) depends on group selection, and hence an individual based explanation accounting for the 2-fold cost of males is needed.

These two influential books, together with a notable contribution by Bell (1982) stimulated the field, which resulted with a vast number of proposed explanations: the review of Kondrashov (1993) includes more than 20 theories for the maintenance of sex. A prominent contribution to testing these theoretical models was made by Antonovics and coworkers, who conducted a series of experiments aimed at testing crucial assumptions and predictions of different ecological hypotheses (Antonovics and Ellstrand 1984; Ellstrand and Antonovics 1985; Schmitt and Antonovics 1986a; Schmitt and Antonovics 1986b; Kelley et al. 1988). These results, together with the series of studies by Lively and coworkers (Lively 1987, 1992; Dybdahl and Lively 1995; Jokela and Lively 1995) lead the scientists to largely abandon the Tangled Bank hypothesis and focus on the Red Queen theory.

The empirical tests of the various genetic/mutational models have also been conducted in 1990's (reviewed in: Rice 2002), focusing primarily on the mutational deterministic hypothesis (Kondrashov 1988), which at the time best resisted the empirical tests. The mutational and ecological hypotheses have been viewed as competing explanations, and the pluralistic approach proposed by West et al. (1999) stimulated a number of responses. Kondrashov's contribution in this discussion illustrates what essentially is the ultimate goal of the research in this field (and the goal of science in general): to find a parsimonious explanation which is general, but also precise, and realistic:

*"I do not like this possibility because such a beautiful phenomenon as sex deserves a nice, simple explanation and messy interactions of very different processes would spoil the story.*

*Of course, this does not mean that such interactions are not, nevertheless, essential."* (Kondrashov 1999).

Yet, maximizing all three of these scientific virtues (generality, precision and realism) in a single study is not possible, as they constitute an inherent trade-off (Levins 1966; Morin 1998; Meirmans and Strand 2010).

The contribution of my study can be viewed as extending the precision and realism of our current understanding of the models explaining the maintenance of sex in nature.

I primarily aimed to assess the extent to which asexual lineages actually realize the predicted two-fold cost reproductive advantage. In Chapter IV we used an approach of a field experiment, to obtain the most direct and relevant estimate of fitness of sexual and asexual lineages in the natural environment. We confirmed the results of the previous study that the sexual *P.antipodarum* indeed pay the cost of males, and that the fecundity of the clones does not correlate with their frequency in a random sample, which implies that additional processes are required for the explanation of the coexistence of sexual and asexual lineages in this population. We were also able to demonstrate that the fitness of clones is seems habitat dependent, which stresses the importance of ecological processes and spatial heterogeneity for the explanations of the maintenance of sex.

In Chapter III, we empirically tested a prediction of the Red Queen hypothesis, that the temporal dynamics of the asexual populations should be higher where the parasite pressure is higher. We found more rapid clonal turnover in the presence of parasites, thus our findings provide support for the Red Queen hypothesis. Analyses of temporal patterns are rare, due to limited availability of long term datasets. These results emphasize the importance of temporal variation, and demonstrate that the temporal scale at which the processes maintaining sex need to and can be explained maybe short, even a single digit number of generations.

In Chapters I and II, we focused on understanding the extent, source of origin and genetic diversity of asexual lineages. We characterized the variation among asexual lineages in ploidy level, genome size and examined their evolutionary history with both nuclear and mitochondrial markers. Although these studies are mostly descriptive, they expand our understanding of what is perhaps the one of the best empirical systems available for studying the maintenance of sex in nature. The exact mechanism responsible for the emergence of

asexual lineages still remains unclear, however the widespread mitonuclear discordance points the future research into explaining the role of mitochondria-related factors on the emergence of new asexal lineages, their fitness and contrasting the hypotheses on the spontaneous vs. infectious origins of new asexual lineages in *P. antipodarum*. It will be interesting to see if these processes that are important for the origin of asexual lineages have any influence on the processes that maintain sexual reproduction, for example, if origin of asexuals have any direct bearing on the polymorphism of resistance genes that govern the Red Queen dynamics.

With the development in the field of molecular genetics, new methods become available also to non-model organisms (e.g. the availability of new sequencing methods makes the inference of asexual lineage age and mutation load possible). Therefore more possibilities for validating the interplay of ecological and mutational hypotheses will be emerging (for existing examples see : Killick et al. 2006; Bruvo et al. 2007). This will hopefully get us closer to obtaining a realistic explanation for the maintenance of sex.

**Literature cited**

Antonovics, J., and N. C. Ellstrand. 1984. Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. Evolution 38:103-115.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Univeristy of California Press, Berkeley (CA).

Bruvo, R., H. Schulenburg, M. Storhas, and N. K. Michiels. 2007. Synergism between mutational meltdown and Red Queen in parthenogenetic biotypes of the freshwater planarian Schmidtea polychroa. Oikos 116:313-323.

Dybdahl, M. F., and C. M. Lively. 1995. Host-parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). Proceedings of the Royal Society of London B, Biological Sciences 260:99-103.

Ellstrand, N. C., and J. Antonovics. 1985. Experimental studies on the evolutionary significance of sexual reproduction. II. A test of the density-dependent selection hypothesis. Evolution 39:657-666.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Jokela, J., and C. M. Lively. 1995. Parasites, sex, and early reproduction in a mixed population of freshwater snails. Evolution 49:1268-1271.

Kelley, S. E., J. Antonovics, and J. Schmitt. 1988. A test of the short term advantage of sexual reproduction. Nature 331:714-716.

Killick, S. C., A. M. Carlsson, S. A. West, and T. J. Little. 2006. Testing the pluralist approach to sex: the influence of environment on synergistic interactions between

mutation load and parasitism in Daphnia magna. Journal of Evolutionary Biology:1603-1611.
Kondrashov. 1999. Being too nice may be not too wise. J Evolution Biol 12:1031-1031.
Kondrashov, A. S. 1988. Deleterious Mutations and the Evolution of Sexual Reproduction. Nature 336:435-440.
Kondrashov, A. S. 1993. Classification of Hypotheses on the Advantage of Amphimixis. J Hered 84:372-387.
Levins, R. 1966. Strategy of model building in population biology. Am. Scientist 54:421-&.
Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519-521.
Lively, C. M. 1992. Parthenogenesis in a freshwater snail: reproductive assurance versus parasitic release. Evolution 46:907-913.
Maynard Smith, J. 1971. The origin and maintenance of sex. Pp. 164-175 *in* G. C. Williams, ed. Group Selection. Aldine-Atherton, Chicago.
Maynard Smith, J. 1978. The evolution of sex. Cambridge Univeristy Press, Cambridge.
Meirmans, S., and R. Strand. 2010. Why Are There So Many Theories for Sex, and What Do We Do with Them? J Hered 101:S3-S12.
Morin, P. J. 1998. Realism, precision and generalityin experimental ecology *in* W. J. Resetarits, and J. Bernardo, eds. Experimental ecology: Issues and pespectives. Oford University Press, New York.
Muller, H. J. 1932. Some genetic aspects of sex. Am Nat 66:118-138.
Muller, H. J. 1964. The Relation of Recombination to Mutational Advance. Mutation Research 1:2-9.
Rice, W. R. 2002. Experimental tests of the adaptive significance of sexual reproduction. Nature Review of Genetics 3:241-250.
Schmitt, J., and J. Antonovics. 1986a. Experimental studies of the evolutionary significance of sexual reproduction. III. Maternal and paternal effects during seedling establishment. Evolution 40:817-829.
Schmitt, J., and J. Antonovics. 1986b. Experimental studies of the evolutionary significance of sexual reproduction. IV Effect of neighbour relatedness and aphid infestation on seedling performance. Evolution 40:830-836.
Weismann, A. 1904. The evolution theory. Edward Arnold, London.
West, S. A., C. M. Lively, and A. F. Read. 1999. A pluralist approach to sex and recombination. J Evolution Biol 12:1003-1012.
Williams, G. C. 1975. Sex and Evolution. Princeton University Press, Princeton (NJ).

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