Genetic distance between species predicts novel trait expression in their hybrids

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ABSTRACT

Interspecific hybridization can generate transgressive hybrid phenotypes with extreme trait values exceeding the combined range of the parental species. Such variation can enlarge the working surface for natural selection, and may facilitate the evolution of novel adaptations where ecological opportunity exists. The number of quantitative trait loci fixed for different alleles in different species should increase with time since speciation. If transgression is caused by complementary gene action or epistasis, hybrids between more distant species should be more likely to display transgressive phenotypes. To test this prediction we collected data on transgression frequency from the literature, estimated genetic distances between the hybridizing species from gene sequences, and calculated the relationship between the two using phylogenetically controlled methods. We also tested if parental phenotypic divergence affected the occurrence of transgression. We found a highly significant positive correlation between transgression frequency and genetic distance in eudicot plants explaining 43% of the variance in transgression frequency. In total, 36% of the measured traits were transgressive. The predicted effect of time since speciation on transgressive segregation was unconfounded by the potentially conflicting effects of phenotypic differentiation between species. Our analysis demonstrates that the potential impact hybridization may have on phenotypic evolution is predictable from the genetic distance between species.

KEYWORDS: adaptive evolution, comparative method, genetic distance, hybridization, transgressive segregation, speciation.

INTRODUCTION

The recombination of genetic material among lineages with divergent evolutionary histories can give rise to novel phenotypes. For more than ten thousand years, since the beginning of domestication of plants and animals, humans have made explicit use of this. Despite influential early publications (Anderson 1949; Anderson and Stebbins 1954; Stebbins 1959; Lewontin and Birch 1966; Stebbins 1966; Templeton 1981) the role of hybridization in evolution, certainly of animals, had for many years received only limited attention by evolutionists. This has recently begun to change. It is now clear that hybridization between species is much more common than was thought previously (Grant and Grant 1992; Arnold 1997; Dowling and Secor 1997; Rieseberg et al. 1999; Barton 2001; Seehausen 2004; Mallet 2007; Schwenk et al. 2008). There is also convincing evidence for that hybridization may facilitate adaptive evolution within species (Grant and Grant 2008) and that it may lead to evolutionary novelty, i.e. to the emergence of novel adaptations and new species, both in

plants (Lexer et al. 2003b) and animals (Schliewen and Klee 2004; Gompert et al. 2006; Mavarez et al. 2006). Some adaptive radiations may have been fuelled by hybridization between distantly related species in plants (Barrier et al. 1999), animals (Feder et al. 2003; Seehausen et al. 2003; Joyce et al. 2005; Mallet 2007) and prokaryotes (Vernikos et al. 2007). Much of this paradigm shift has been driven by developments in molecular genetics that made it possible to identify hybrid individuals (Rieseberg and Linder 1999; Anderson and Thompson 2002) and lineages (Ungerer et al. 1998) more easily and track the traces of reticulate evolution with more confidence (Marri et al. 2007).

Next to their intrinsic fitness, the evolutionary potential of hybrid populations depends on the ecological competitiveness of hybrid genotypes. Simulation models (Buerkle et al. 2000) and experiments (Abbott 1992; Jackson and Tinsley 2003; Lexer et al. 2003a) suggest that hybrid populations are likely to persist only if they can occupy previously underutilised fitness peaks on the local adaptive landscape. However, in most cases hybrids resemble one of the parents or express intermediate trait values that lay between the parental means. Intermediate hybrid phenotypes are not likely to persist without spatial isolation from the parents (Barton and Hewitt 1985) unless an underutilized fitness peak requires intermediate trait values (Mallet 2007). Yet, hybrids frequently express trait values exceeding the range between the parental means, which is referred to as transgressive segregation (Slatkin and Lande 1994; Rieseberg et al. 1999). Phenotypes are transgressive if they lie outside the phenotypic range of both parental species. Theoretically, transgressive traits can provide hybrid genotypes with novel adaptive potential, not shared by either parental population. Populations of such hybrid genotypes may then diverge from the parental species through the same mechanisms that play a role in classical ecological speciation (Seehausen 2004). Ecological hybrid speciation facilitated by transgressive segregation has been demonstrated in detail in hybrid sunflower species (Schwarzbach et al. 2001; Lexer et al. 2003b; Rieseberg et al. 2003).

Transgressive segregation is common and widespread. Rieseberg at al. (1999) found evidence for transgressive segregation in 110 of 113 studies on hybridizing plant species, and in 45 of 58 cases of hybridizing animal species. They further found that 59% of the 579 investigated traits in plants, and 31% of the 650 traits in animals, were transgressive. Several different mechanisms have been proposed to explain how the rearrangement of genomes can create phenotypic novelty (Rick and Smith 1953; Grant 1975; DeVicente and Tanksley 1993; Monforte et al. 1997; Rieseberg et al. 1999). A widely accepted view is that transgression is the result of the recombination of alleles at quantitative trait loci (QTL), that are fixed for

alleles of opposite sign in the parents which sum up to an extreme trait value when recombined in their hybrids a mechanism commonly referred to as complementary gene action. While parental phenotypes are constrained to a certain trait value range (because each parent fixed counteracting alleles at different QTLs for the same trait), some of their hybrids can inherit complementary alleles from both parents, generating transgressive hybrid phenotypes. Although non-additive effects by overdominance (in which the combination of divergent alleles at a particular locus endows the heterozygote with a more extreme trait value than both homozygotes) and epistatic interactions (the action of one gene is modified by one or several other genes) may contribute, quantitative genetic studies on plant hybrids consistently identified complementary gene action as the primary cause of transgression (Weller et al. 1988; De Vicente and Tanksley 1993; Mansur et al. 1993; Clarke et al. 1995; Ecke et al. 1995; Li et al. 1995; Monforte et al. 1997; Bradshaw et al. 1998; Kim and Rieseberg 1999; Rieseberg et al. 2003).

Given a purely additive regime, transgression due to complementary gene action can only be observed in the F2 and higher hybrid generations. In the F1 generation, additive effects only produce intermediate phenotypes. However, if dominance prevails at some loci contributing to complementary gene action, transgressive phenotypes can already occur in F1 hybrids. Dominance produces extreme trait values in the F1 generation because hybrid individuals express only the dominant allele at all heterozygous loci, and so end up expressing fewer alleles with antagonistic effects on different loci than their homozygous counterparts. For this, parental species must be recessive homozygotes for at least one locus, and it must be a different locus in each parental species (e.g. the diploid two-locus two-allele parental genotypes A_bb and aaB_ (each with trait values of 0) can produce A_B_ or aabb F1 hybrids with transgressive trait values of +2 or -2, respectively).

We predicted that if some of the transgression in interspecific F1 hybrids is caused by complementary gene action or epistasis, its frequency should correlate positively with the genetic distance between hybridizing species, because the number of loci at which two different species have fixed alleles with opposite sign should increase with time since speciation. To test this we collected data on the frequency of transgressive segregation in hybrids from published work, and molecular sequence data for the same species from GenBank. We calculated pairwise sequence differences between hybridizing species. We then mapped these and transgression frequency on published phylogenetic trees. Finally we calculated independent contrasts (Felsenstein 1985) in genetic distance and in transgression

frequency between pairs of hybridizing species for a test of the predictions that is controlled for phylogenetic non-independence.

Variation in the extent of phenotypic differentiation between the parental lines can potentially confound the predicted relationship between genetic distance and transgression. Phenotypically similar species are more likely to produce transgressive hybrid offspring than dissimilar species. This is because the maintenance of phenotypic similarity despite proceeding genetic divergence requires the accumulation and fixation (by stabilizing selection) of antagonistic allelic effects independently within the two species (DeVicente and Tanksley 1993; Mansur et al. 1993; Kim and Rieseberg 1999). Hence, two similar species that have experienced stabilizing selection on the same traits are likely to eventually fix different alleles at some QTLs, which would then cause transgression when these are recombined in hybrids. Conversely, phenotypically divergent species are less likely to produce transgressive offspring as the genetic basis for complementary gene action may be missing because of the fixation of alleles with opposite signs on loci with a consistent directional selection history. To test if phenotypic divergence, besides genetic distance, also affected the occurrence of transgression, we calculated an index of parental phenotypic divergence for each of the traits included in our analysis.

METHODS

Literature Search

All cases used in our analysis were identified in a search using Web of Science (http://portal.isiknowledge.com/portal.cgi) with the keyword combination "interspecific hybrid* AND morpholog*" (965 hits). From this literature we selected studies that met the following criteria: (1) Finding transgressive traits for breeding purposes was not the aim of the study. (2) Data from wild hybrids were included only if their hybrid identity was confirmed with molecular markers. (3) Data were present for at least three different traits. Trait ratios (e.g. leaf width / leaf length) were excluded, except if neither numerator nor denominator were included separately. (4) Data had to be quantitative. We excluded qualitative data (e.g. illustrations of leaf shapes, description of flower colouration). (5) To obtain a comparable measure of genetic distance between species, we used the same gene for all species. This required availability of sequence data on NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) (for details see below). (6) Sequences had to be longer than 500 bp.

A total of 62 plant hybrid systems met our criteria, comprising a multitude of taxa (47 different eudicot crosses, 12 monocot crosses, 2 crosses within magnoliids and one within *Nymphaeaceae*; Table 1; Supplementary Fig. 1). In addition, we were able to collect a small data set on animal species comprising 15 hybrid systems, of which 12 were Teleost fish, of which again 8 crosses within the Teleost family *Cyprinidae*.

We first ran all analyses exclusively on F1 before including the cases where only data on BC (hybrids backcrossed to one or both of the parents), F2, F3 or wild hybrids were available (Table 1). The detectability of transgression in our analysis therefore was mainly limited to cases involving loci with heterozygous effects or dominant alleles, the complementation among which is visible in the heterozygous F1 hybrids.

Assessment of transgression frequency

We collected all available phenotypic data from published articles, including morphological, physiological and life history traits of both parental species and their hybrid offspring. Character means that lay outside the range between the means of both parental species in a negative or positive direction were defined as transgressive. Where only phenotypic ranges were given, but no mean values, we considered hybrids as transgressive if part of their trait value range fell outside of the combined parental ranges. Where means and ranges were given, we only scored those traits as transgressive that had hybrid means outside of the range of the two parental means, regardless of the distribution of the trait ranges, which is conservative with regard to our expectation. Hybrid means can fall between the parental means while the hybrid range can still exceed the parental trait range. We then calculated the ratio between the number of traits that were transgressive to the total number of traits that were measured (hereafter this ratio will be referred to as 'transgression frequency').

Assessment of genetic distance

To obtain genetic distances for all parental species pairs, uncorrected p-distances (Takahashi and Nei 2000; Nei and Kumar 2003) were calculated from gene sequences taken from NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank/). Calculating genetic distances on the basis of other substitution models (e.g. Jukes-Cantor, Kimura 2- parameter) did not affect the results of our analyses.

As the common currency for measuring interspecific divergence (Chapman and Burke 2007) we used the internal transcribed spacer region (ITS I and II) for plants and cytochrome b for animals. Between 1 and 10 sequences per parental species (depending on their

availability on GenBank) were aligned in ClustalW (Thompson et al. 1997) and alignments were manually optimised. Genetic distances were calculated in MEGA 4 (Kumar et al. 2004). If multiple sequences available were for a pair of species, we calculated the average of all possible pairs of sequences. In four cases (*Eucalyptus, Dianthus, Cerastium* and *Piper*), where sequences for one of the two parental species of a cross were missing, we calculated the average genetic distance between the available parental species and all other species of the genus for which sequences were available. Further, to test if these averaged distances affected our tests, we re-calculated all analyses without these four taxa and compared the results to those of the complete data set.

Chi-square tests of homogeneity of base pair frequencies calculated in PAUP* 4.0b10 (Swofford 2001) revealed no significant heterogeneity between the hybridized species pairs ($p > 0.05$ in all cases).

Assessment of phenotypic differentiation

Phenotypic differentiation was calculated by dividing the absolute trait value difference between the two parental species of any cross by the larger of the two trait values, resulting in an index ranging from 0 (no trait differentiation) to 1 (large trait differentiation). This was done for each trait reported per hybridized species pair. Logistic regression was used to test transgressive segregation as binary response variable against differentiation index, running a separate regression analysis for each hybrid system. A one-sample t-test on the slopes from all regression lines was used to assess if they significantly differed from zero.

We also tested if genetic distance was correlated to the degree of phenotypic differentiation by calculating linear regressions of the phenotypic differentiation index of all traits across all hybrid systems against genetic distance.

Calculating Independent Contrasts

Independent contrasts (Felsenstein 1985; Pagel 1999) in p-distances and in transgression frequency were calculated for all pairs of hybridized species (note that the terminal taxa in this analysis are pairs of species, rather than species) and for all superior nodes deeper in the phylogeny down to the pair of nodes right above the last common ancestor of two species pairs. We then used standard regression techniques in JMP 7 (SAS Institute) to estimate the relationship between transgression frequency and genetic distance. This method is equivalent to the phylogenetically weighted averaging procedure that has been used in similar contexts

(Fitzpatrick 2002; Bolnick and Near 2005). This procedure is required to control for any phylogenetic inertia in transgression frequency. At the same time it ensures the statistical independence of data points (Harvey and Pagel 1991). Phylogenies were taken from The Angiosperm Phylogeny Group (The Angiosperm Phylogeny Group 2003) and from the Tree of Life project (Maddison and Schulz 1996-2007) (Supplementary Fig. 1).

In the regression analysis, we used Δ genetic distance as the independent variable and Δ transgression frequency as the dependent variable. The data were standardized, i.e. each variable was centred to mean zero by subtracting the mean and dividing by the standard deviation. Both variables were normally distributed, confirmed with Shapiro-Wilkinson tests for normality. The y-intercept of all regression lines was constrained to the origin. This was necessary because when calculating contrasts, the direction of subtraction between the two values of any variable is random and hence the sign of the contrast is arbitrary as long as the contrasts in the two variables that are tested are calculated by subtraction in the same direction (Garland et al. 1992).

For plants, we used six different levels of phylogenetic inclusiveness in our analysis, gradually climbing down the phylogenetic tree from the tips to the root. The first regression (regression I) contained only contrasts calculated between species within genera (e.g. *Trifolium alexandrinum* x *T. resupinatum* versus *Trifolium repens* x *T. ambiguum*). If a specific hybrid cross was studied in more than one publication, we calculated the average of the transgression frequencies from all studies before applying contrasts. The second regression (regression II) contained all within-genus contrasts again, plus contrasts calculated between genera within families (e.g. within *Fabaceae*: *Trifolium* versus *Medicago*). All genetic distances and transgression frequencies were averaged within genera before calculation of the contrasts. We did not perform a separate analysis on the between-family within-order level because only in two eudicot and two monocot cases did we have data on more than one family within an order, which added only little extra information to the previous regression analysis. The third regression (regression III) hence contained the within-genus and within-family contrasts plus contrasts calculated between orders within the next 'supraordinal' clades (e.g. within *Fabids: Fabales* versus *Malphigiales*). Again, all values were averaged beforehand within orders. The fourth regression (regression IV) was calculated as described above containing all previously calculated contrasts plus contrasts calculated within the next more inclusive taxonomic grouping deeper down towards the root of the tree (e.g. within *Rosids*: *Fabids* versus *Malvids*). The same procedure was applied to calculate the fifth regression containing all contrasts within eudicots (regression V) and monocots,

respectively. The sixth regression (regression VI) contained all contrasts within angiosperms. No suitable data was available for gymnosperms.

For animals we used the same taxonomic levels of analysis with the difference that contrasts were only available for regressions II, III and VI.

Finally, to test whether transgression frequency was affected by the number of phenotypic traits reported, we conducted a regression analysis of transgression frequency on the total number of traits.

RESULTS

Analysis of the 62 plant studies examined here, reporting on phenotypic variation in segregating hybrid populations and their respective parental populations, resulted in 36% transgressive traits (249 traits out of a total of 687 traits). An earlier study found as much as 59% transgressive traits in a large survey on plant hybrid systems (Rieseberg et al. 1999).
Analysis of the 15 animal studies resulted in 29% transgressive traits (65 traits out of a total of 222 traits). This frequency of transgression is in close agreement with an earlier study that found 31% transgressive traits in animal hybrids (Rieseberg et al. 1999). Only 14 % of the studies analysed by us where also included in that earlier study, while 86% of our data were not analysed in this way before.

There was no correlation between the number of traits reported and the proportion of transgressive traits within either eudicots ($R^2 = 0.01$, $F_{1,46} = 0.24$, $p = 0.63$), monocots ($R^2 = 0.0$, $F_{1,11} = 0.06$, $p = 0.81$), animals ($R^2 = 0.0$, $F_{1,14} = 0.0$, $p = 0.99$), or the combined data set ($R^2 = 0.01$, $F_{1,77} = 0.56$, $p = 0.46$).

The frequency of transgressive traits increased significantly with increasing genetic distance in eudicot plants. The relationship was particularly strong in the phylogenetically least inclusive comparisons, when only contrasts between pairs of species within genus were considered (regression I, only F1 hybrids: $R^2 = 0.57$, $F_{1,17} = 21.5$, $p < 0.001$; all hybrids: $R^2 = 0.43$, $F_{1,26} = 18.72$, $p < 0.001$; Fig. 1a). Contrasts from one study were excluded from this analysis because they represented outliers from the distribution (i.e. they fell outside of the upper and lower quartile ± 1.5 * interquartile range). In this study, Bletsos *et al.* (Bletsos et al. 2004) produced interspecific hybrids between the eggplant species *Solanum melongena* and *S. macrocarpon*. Two of the three contrasts in genetic distance between this species pair and other *Solanum* crosses, were unusually high while the associated contrasts in transgression frequency were low (genetic distance / transgression frequency: -0.062 / -0.111 and -0.083 / -0.305). When this study was included, the predictive power of genetic distance decreased but

the regression slope remained highly significant (regression I, only F1 hybrids $R^2 = 0.5$, $F_{1,20} = 18.92$, $p < 0.001$).

Interestingly, when contrasts between more inclusive nodes in the phylogeny of angiosperms (regressions II-VI) were included, the signal became successively weaker. The fit between transgression frequency and genetic distance was slightly less tight when contrasts between genera of the same family were added (regression II, only F1 hybrids: $R^2 = 0.41$, $F_{1,24} = 15.78$, $p < 0.001$; all hybrids $R^2 = 0.28$, $F_{1,43} = 14.48$, $p < 0.001$; Fig. 1b). The signal decreased further when contrasts between orders were added (regression III, only F1 hybrids: $R^2 = 0.30$, $F_{1,33} = 13.06$, $p = 0.001$; all hybrids: $R^2 = 0.25$, $F_{1,48} = 14.95$, $p < 0.001$; Fig. 1c), and then remained little changed when contrasts between 'supraordinal' clades (regression IV, only F1 hybrids: $R^2 = 0.24$, $F_{1,37} = 16.76$, $p < 0.001$; all hybrids: $R^2 = 0.27$, $F_{1,54} = 19.95$, $p < 0.001$; Fig. 1d) and contrasts within all eudicots (regression V, only F1 hybrids: $R^2 = 0.21$, $F_{1,40} = 17.64$, $p < 0.001$; all hybrids: $R^2 = 0.28$, $F_{1,57} = 21.45$, $p < 0.001$; Fig. 1e) were added. When we added monocots and analysed all contrasts within angiosperms, i.e. including contrasts from all taxonomic levels of both eudicots and monocots, the signal was abruptly lost altogether (regression VI, only F1 hybrids: $R^2 = 0.11$, $F_{1,51} = 2.04$, $p = 0.158$; all hybrids: $R^2 = 0.00$, $F_{1,76} = 0.08$, $p = 0.77$; Fig. 1f).

Inclusion or exclusion of the four crosses, where sequences for one of the two parental species of a cross were missing and for which we calculated averaged genetic distances between the available parental species and all other species of the respective genera *(Eucalyptus, Dianthus, Cerastium, Piper*), had little effect on the results (results shown only for F1 hybrids after excluding *Eucalyptus, Dianthus, Cerastium* and *Piper*: regression I, $R^2 = 0.58$, $F_{1,16} = 20.32$, $p = 0.001$; results of regression II and III remained unchanged, regression IV, $R^2 = 0.35$, $F_{1,36} = 18.62$, $p < 0.001$; regression V, $R^2 = 0.34$, $F_{1,37} = 18.33$, $p < 0.001$; regression VI, $R^2 = 0.12$, $F_{1,47} = 1.96$, $p = 0.168$). Hence, the averaging of genetic distance within genera did not bias our results.

The monocot data gave different results. The slopes of almost all regressions were negative but none was significant (regression I, only F1 hybrids: $R^2 = -0.25$, $F_{1,3} = 0.67$, $p = 0.49$; all hybrids: $R^2 = -0.04$, $F_{1,4} = 0.12$, $p = 0.75$; regression II, only F1 hybrids: $R^2 = -0.25$, $F_{1.4} = 1.0$, $p = 0.39$; all hybrids: $R^2 = -0.26$, $F_{1,11} = 3.53$, $p = 0.09$; regression IV, only F1 hybrids: $R^2 = -0.41$, $F_{1,6} = 3.5$, $p = 0.12$; all hybrids: $R^2 = -0.22$, $F_{1,13} = 2.66$, $p = 0.128$; regression V, only F1 hybrids: $R^2 = -0.02$, $F_{1,9} = 0.14$, $p = 0.71$; all hybrids: $R^2 = -0.19$, $F_{1,16} = 2.97$, $p = 0.11$; regression VI, only F1 hybrids: $R^2 = 0.02$, $F_{1,10} = 0.16$, $p = 0.7$; all hybrids: $R^2 = -0.17$, $F_{1,19} = 2.97$, $p = 0.1$). Regression III could not be calculated because our data set

contained no monocot hybrid crosses for contrasts between orders of the same 'supraordinal' clade.

Surprisingly, the animal data set produced significant negative slopes at all levels of phylogenetic inclusiveness (regression II, only F1 hybrids: $R^2 = -0.58$, $F_{1,10} = 12.11$, $p = 0.007$, all hybrids: $R^2 = -0.45$, $F_{1,22} = 16.79$, $p < 0.001$, Fig. 2a; regression III, only F1 hybrids: $R^2 = -0.40$, $F_{1,14} = 8.8$, $p = 0.011$, all hybrids: $R^2 = -0.30$, $F_{1,32} = 8.23$, $p = 0.007$, Fig. 2b; regression VI, only F1 hybrids: $R^2 = 0.47$, $F_{1,15} = 12.33$, $p = 0.004$, all hybrids: $R^2 = -0.21$, $F_{1,35} = 8.95$, $p = 0.005$, Fig. 2c).

In plants, 41 hybrid systems out of 59 (3 systems were excluded here because phenotypic data was only provided as range and not as mean in the source paper) showed a negative correlation between the phenotypic trait differentiation of the parental species and the occurrence of transgression in hybrids (Fig. 3a). Twelve of these 41 negative regression lines were significant, of which four remained significant after sequential Bonferroni correction. Ten systems had regression lines equal to zero and only eight systems showed positive trends of which none was significant. In animals, 10 hybrid systems out of 15 showed a negative correlation between phenotypic differentiation and transgression frequency, four of which were significant (Fig. 3b). None of the animal systems showed a positive trend. A one-sample t-test revealed that, on average, the slopes were significantly different from zero (plants: $t_{59} = -5.04$, $p < 0.001$; animals: $t_{15} = -2.29$, $p = 0.038$).

In the animal data set, the phenotypic differentiation of the parental species increased significantly with genetic distance ($R = 0.7$, $F_{1,219} = 17.86$, $p < 0.001$) using phenotypic data from each trait across all hybrid systems. The same analysis did not reveal a significant relationship in plants ($R = 0.0$, $F_{1,644} = 0.01$, $p = 0.93$).

DISCUSSION

The occurrence of phenotypic novelty through interspecific hybridization is common (Rieseberg et al. 1999) and has been suggested to be a potentially important source of adaptive genetic variation where ecological opportunity exists (Harini and Ramachandra 2003; Lexer et al. 2003b; Johnston et al. 2004; Seehausen 2004; Albertson and Kocher 2005). We predicted, based on the previous finding that transgression in hybrids is often caused by complementary gene action or epistasis (Rieseberg et al. 1999; Rieseberg et al. 2003), that the frequency of transgression should positively scale with the genetic distance between the hybridizing species. We calculated independent contrasts (Felsenstein 1985) between species

pairs in genetic distance and in the proportion of transgressive traits in their hybrids to test the predicted relationship using phylogenetically controlled regressions.

Our data on 47 eudicot plant hybrid systems is consistent with our prediction. The correlation between transgression frequency and genetic distance was significantly positive. Using independent contrasts calculated between species of the same genus, more than 40% of the variance in transgression frequency was explained by genetic distance (Fig. 1a).

Inclusion of contrasts between increasingly inclusive clades caused a successive shallowing of the slope and weakening of the correlation. This could partly be an effect of increasingly different genetic architectures between lineages. The latter is supported by our finding of between-lineage variation in the frequency of transgressive phenotypes. For example, the correlation between genetic distance and transgression frequency is much stronger in rosids ($R^2 = 0.79$, $F_{1,12} = 49.96$, $p < 0.001$) than in asterids ($R^2 = 0.24$, $F_{1,16} = 5.23$, $p = 0.037$) when analysed separately on the within-genus level. An unpaired t-test (computed as the difference between the two slopes divided by the standard error of the difference between the slopes) revealed a significant difference between the slopes (unpaired *t*-tests, $t = 17.47$, p (two-tailed) < 0.001).

There is potential for measurement error in all variables we used (genetic distance, phenotypic distance and transgression frequency) deriving from a) variation in the accuracy with which phenotypic traits were reported in the literature, and b) restricting the calculation of genetic distance to only one locus (internal transcribed spacer region I and II), and c) because we assumed a clock-like evolution of this one gene. Given these possible sources of error, it is remarkable that genetic distance explains such a large proportion (> 40%) of the variance in transgression frequency among the phylogenetic contrasts within genera.

Our monocot data suggests a relationship of the opposite direction such that genetically more distant species are less likely to produce transgressive hybrid phenotypes. However, none of the slopes was significant and the strength of the correlations was weaker than in the eudicot data set at all taxonomic levels. The sample size for monocots (n of different species crosses = 12) was much smaller than that for eudicots (n = 44), and it was dominated by *Allium* crosses (see Table 1). It is hence possible that the observed trend, or the absence of any strong trend, is not representative for monocot plants.

Opposite to the signal in eudicots, the animal data revealed a significantly negative correlation between transgression frequency and genetic distance (Fig. 2 a-c). However, as for monocots the taxonomic breadth of this data set was limited and dominated by one group (12 of the 15 studies were on Teleost fish, of which 8 were species crosses within the family

Cyprinidae). Hence, we suggest handling these results with some caution. To be able to make more solid conclusions for animals, a phylogenetically more inclusive sampling is desirable. This was not possible with the data at hand.

Variation in the degree of phenotypic differentiation between parental species is a factor that needs to be taken into account when trying to asses the causes of variation in transgression frequency in interspecific hybrids. The genetic conditions allowing for complementary gene action are more likely given for traits that have been under stabilizing selection in both hybridizing species. Stabilizing selection leads to fixation of QTLs with alternating sign, that are complementary when recombined. Conversely, the probability for the appearance of transgressive hybrid offspring should be low between phenotypically divergent species. In response to divergent selection, each species is likely to have fixed alleles of same sign at multiple QTLs, but the sign being different between the species. Such genetic architecture of species differences would leave little opportunity for complementary gene action in hybrids. Transgressive phenotypes for oral jaw shape were absent amongst the hybrid offspring of two closely related Lake Malawi cichlid species with markedly different jaw morphology (Albertson and Kocher 2005). QTL sign tests implicated divergent directional selection on jaw shape in the two species (Albertson et al. 2003). Therefore, we tested if transgressive segregation frequency in interspecific hybrids was partially determined by phenotypic differentiation of the parental species. We found our prediction strongly confirmed. In both plants and animals, the large majority of hybridizing species pairs showed a negative correlation between the extent of differentiation in a given trait, and the occurrence of transgressive expression of that trait in their hybrids (Fig 3 a, b).

The magnitude of phenotypic differentiation was not predicted by the genetic distance between species in our plant data set. It follows that in plants the predicted effect of time since speciation (genetic distance) on the occurrence of transgressive segregation was unconfounded by the potentially conflicting effects of phenotypic differentiation between species.

In contrast with plants, we found a significant positive relationship between genetic distance and phenotypic differentiation in the animal data. It is hence possible that in animals, the expected positive effect of time since speciation was masked by the expected negative effects of phenotypic differentiation. Relatively large proportions of transgressive traits observed in hybrids between closely related animal species may be a result of relatively little phenotypic differentiation, whereas distantly related species may have shown fewer than

expected transgressive traits because of the relatively larger phenotypic differentiation between them.

The genetic mechanism underlying extreme trait expression can, however, not be conclusively determined from the phenotype distribution alone. If trait values are correlated with fitness, e.g. if certain traits are more strongly expressed in individuals of better constitution, transgression may also result from heterosis. Similarly, the effects of genetic incompatibilities such as Dobzhansky-Muller-interactions can lead to transgression, e.g. if reduced growth leads to smaller trait values in hybrids. Because our data are almost exclusively from first generation hybrids where heterosis is at its maximum, it is possible that increased hybrid fitness caused the expression of transgressive values in some traits. This is, however, unlikely to account for a major part of our results because at larger genetic distances the effects of heterosis on hybrid fitness are counteracted by genetic incompatibilities accumulating with time since speciation, which effectively decreases heterosis in distant crosses (Moll et al. 1965). We hence conclude that an increase in complementary gene action and epistasis are the more likely explanation for the positive relationship between genetic distance and the frequency of transgression we observed.

Since only those hybrid genotypes with heritable transgressive trait values add to the 'working surface' of natural selection, transgression based on heterosis is not expected to lead to the evolution of novel adaptations. Transgressive hybrid genotypes generated by complementary gene action and epistasis on the other hand can breed true and fixation of the multilocus genotype with the most beneficial combination of parental alleles at different loci is possible (Fitzpatrick and Shaffer 2007). However, the functional relevance of the transgressive trait values detected in this analysis is mostly unknown (Lexer et al. 2003a; Gross et al. 2004; Johnston 2004) and our data make no prediction with regard to hybrid fitness. In fact some of the extreme phenotypes reported here may be mal-adaptive. Yet, under some ecological circumstances the increased working surface for selection generated by transgressive segregation in hybrids may well compensate for an average fitness loss through genetic incompatibilities (Hatfield and Schluter 1999; Via 2002), a scenario particularly relevant when novel habitats are colonized or when existing habitats have been thoroughly altered.

We conclude that both time since speciation and phenotypic differentiation have to be taken into account to predict the frequency of phenotypic novelty and the opportunity for adaptive evolution emerging from interspecific hybridization. Future work should compare transgression frequencies in hybrids from controlled crosses between closely and more

distantly related species with both similar and divergent phenotypes. Such analysis should be performed using species of a single evolutionary lineage to avoid the confounding effect of phylogenetic variance in transgression frequency.

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FIGURE CAPTIONS

Supplementary Figure 1: Angiosperm phylogeny used for calculating independent contrasts, modified from APG II (The Angiosperm Phylogeny Group 2003). Genera included in the analysis shown on the right side of the arrows. Number of different species crosses per genus in brackets. Number of same species crosses not shown.

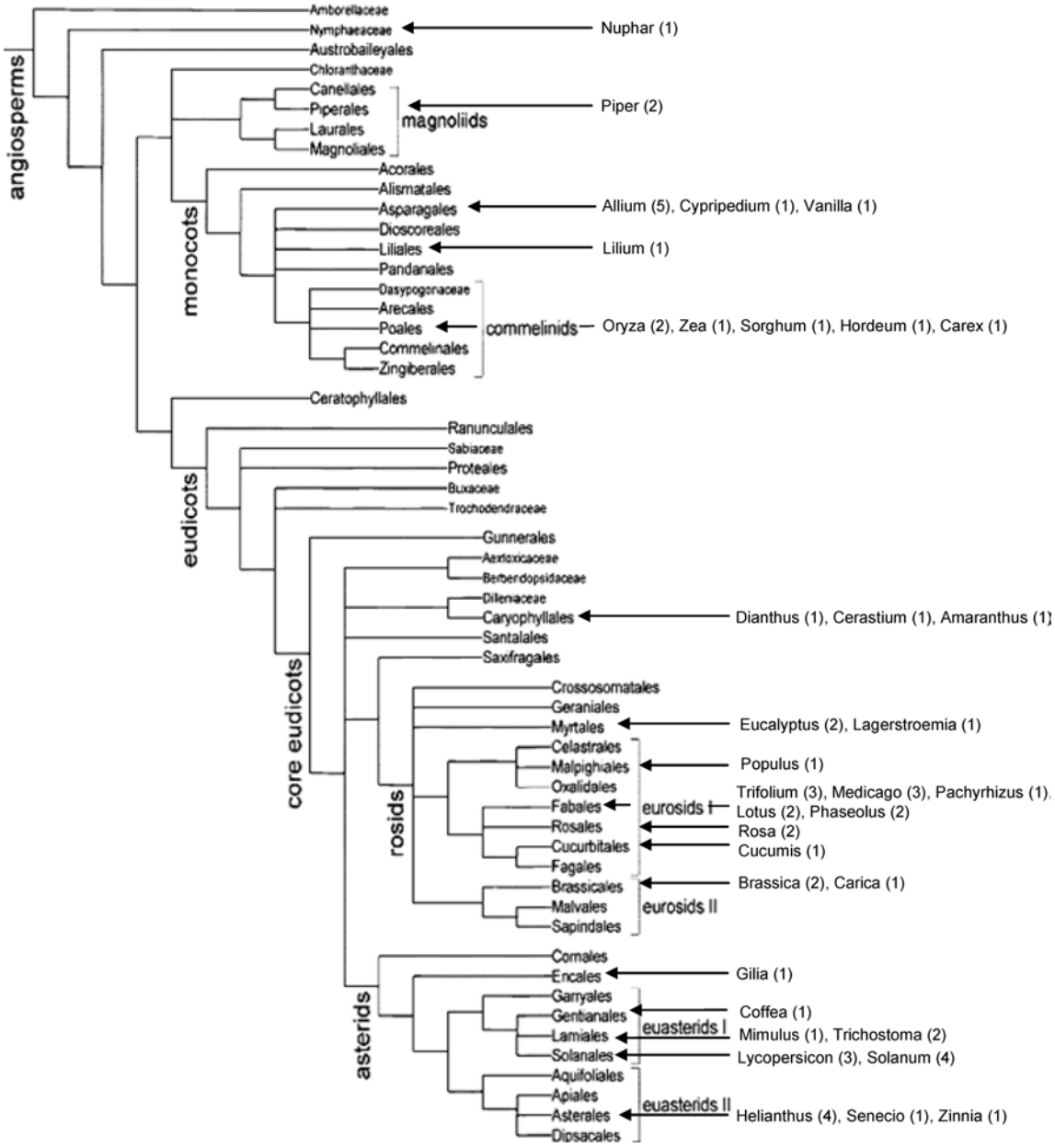

Figure 1 a-f: Linear regressions (I-VI) of transgression frequency on genetic distance (uncorrected p-distance calculated from internal transcribed spacer region I and II sequences) using the eudicot data set. Independent contrast a) between pairs of species within the same genus; b) same as (a) plus contrasts between genera of the same family; c) same as (b) plus contrasts between orders of the same supraordinal clade; d) same as (c) plus contrasts between supraordinal clades within the next higher taxonomic grouping; e) same as (d) plus contrasts within eudicots; f) same as (e) plus contrasts within angiosperms including eudicots and monocots.

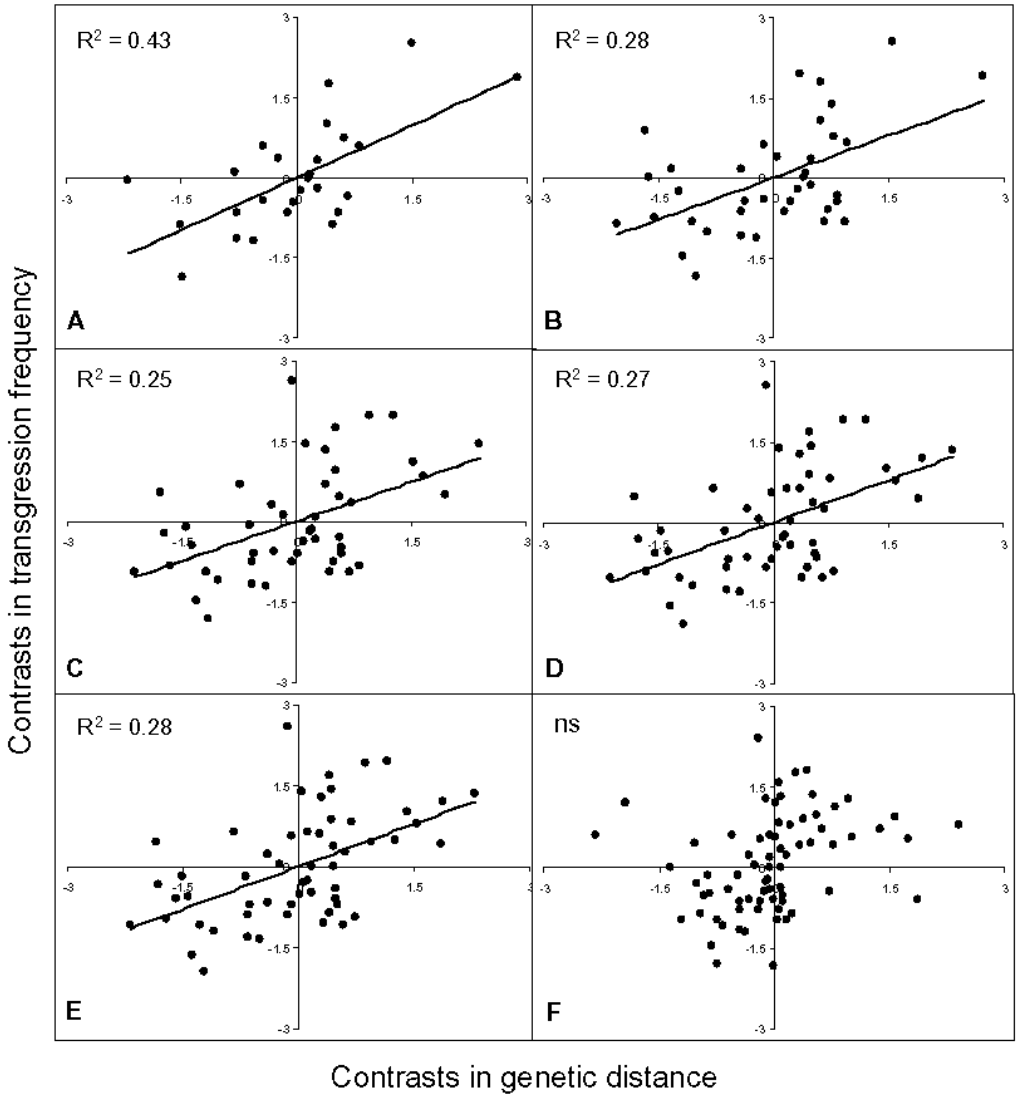

Figure 2 a-c: Linear regressions (II, III, VI) of transgression frequency on genetic distance (uncorrected p-distance calculated from cytochrome b sequences) using the animal data set. Independent contrast a) between genera of the same family; b) same as (a) plus contrasts between orders of the same class; c) same as (b) plus contrasts between classes within phylum.

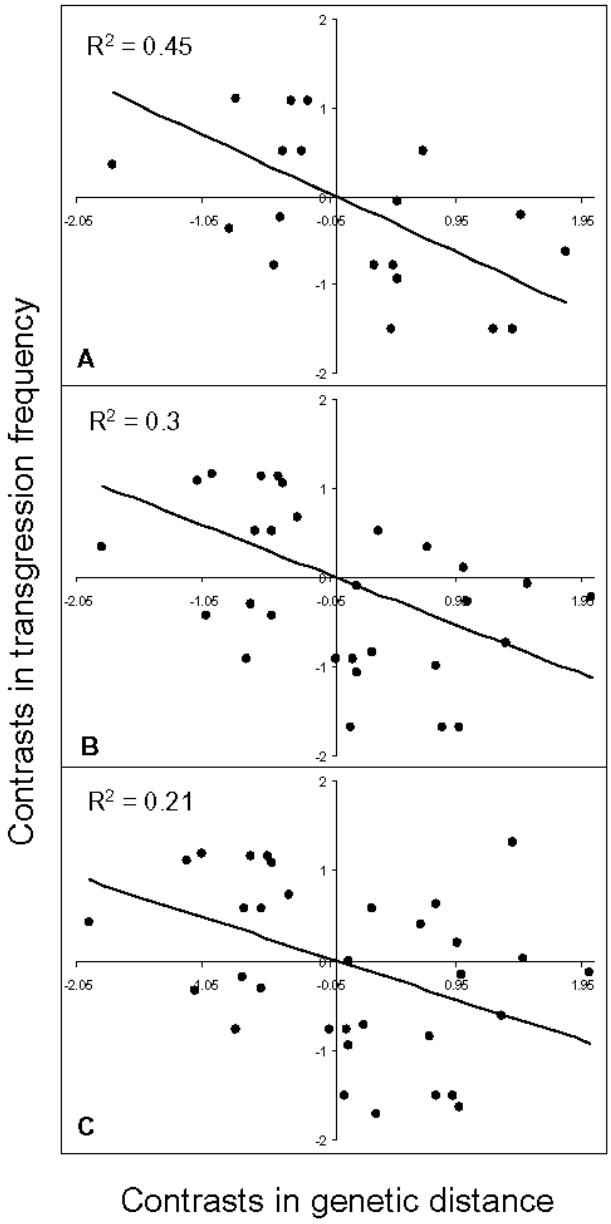

Figure 3 a-b: Logistic regression of occurrence of transgressive segregation against phenotypic differentiation between hybridizing species of a) plants and b) animals. Each regression line represents one pair of hybridizing species. Sample sizes are n = 55 for plants and n = 15 for animals. The thick line shows the average relationship measured across all traits of all hybrid systems.

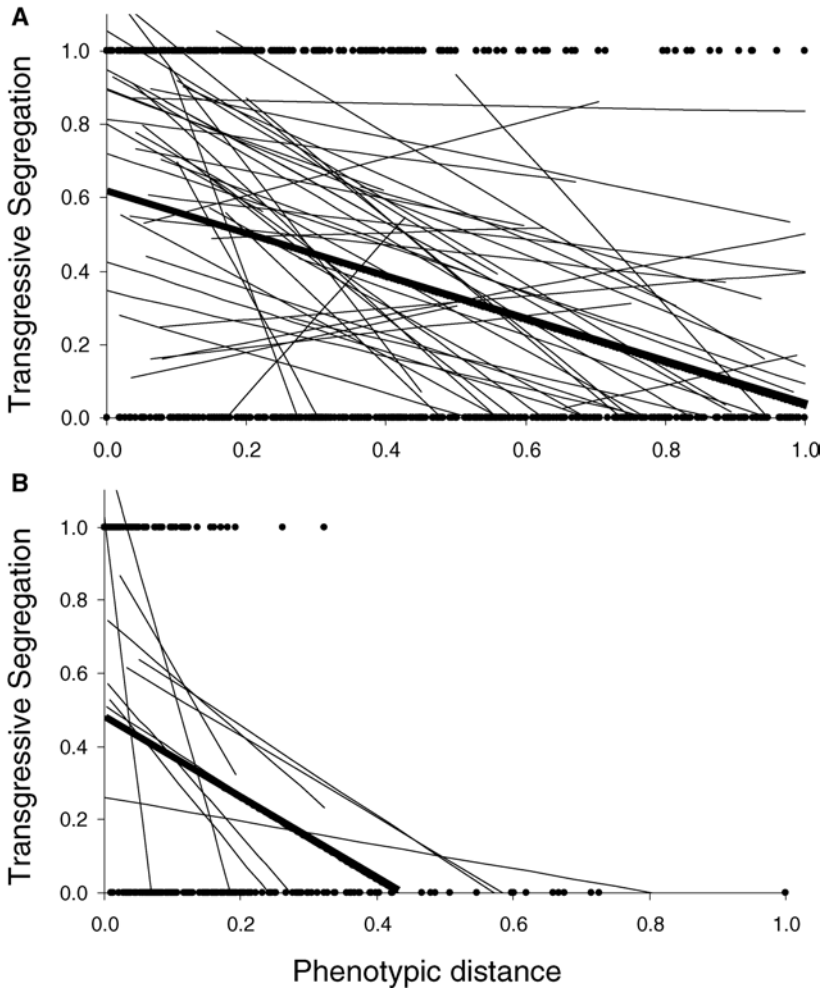

Table 1: The 62 plant and 15 animal hybridized species pairs with genetic distances (uncorrected p-distances calculated from ribosomal DNA sequences ITS 1 and 2 for plants and from cyochrome b for animals), transgression frequencies, number of phenotypic traits assessed, hybrid generation, and the source reference.

hybrid system	genetic distance	trans-gression frequency	n traits	hybrid gene-ration	source reference
PLANTS					
Rosa rubiginosa x R. sherardii	0.032	1	5	F1	G. Werlemark, H. Nybom, Hereditas, 134, 1 (2001)
Rosa sherardii x R. villosa	0.028	0.6	5	F1	G. Werlemark, H. Nybom, Hereditas, 134, 1 (2001)
Trifolium alexandrinum x T. constantinopolitanum	0.009	0.0909	11	F1	A.K. Roy et al, Plant Cell Report, 22, 9 (2004)
Trifolium alexandrinum x T. resupinatum	0.053	0.6667	6	F1	P. Kaushal et al, Plant Cell Tissue and Organ Culture, 83, 2 (2005)
Trifolium repens x T. ambiguum	0.022	0.0667	15	F1	M.T. Abberton et al, Plant Breeding, 117, 5 (1998)
Medicago sativa sativa x M. falcata	0.007	0.2778	18	F1	H. Riday, personal communication
Medicago sativa x M. rugosa	0.028	0.6667	3	F1	Y. Mizukami et al, Plant Cell Tissue and Organ Culture, 84 (2006)
Medicago sativa x M. arborea	0.0245	0.4667	15	F1	E. Nenz et al, Theoretical and Applied Genetics, 93 (1996)
Pachyrhizus tuberosus x P. ahipa	0.0768	0.75	16	F1	W.J. Gruneberg et al, Genetic Resources and Crop Evolution, 50, 7 (2003)
Lotus alpinus x L. conimbricensis	0.088	0.3636	11	F1	L.S. O'Donoghue et al, Canadian Journal of Botany, 68 (1990)
Lotus burtii x L. ornithopodioides	0.074	0.2727	11	F1	L.S. O'Donoghue et al, Canadian Journal of Botany, 68 (1990)
Phaseolus vulgaris x P. acutifolius	0.0628	0.5	6	F3	S. Honma, Journal of Heredity, 47 (1956)
Phaseolus vulgaris x P. lunatus	0.0727	0.75	4	unclear	S. Honma, O. Heeckt, Journal of Heredity, 50 (1959)
Populus trichocarpa x P. deltoides	0.0085	0.2	25	F1	R. Wu et al, American Journal of Botany, 84, 2 (1997)
Populus trichocarpa x P. deltoides	0.0085	0.2083	8	F1	R. Wu, R.F. Stettler, Heredity, 81 (1998)
Cucumis sativus x C. hystrix	0.04	0	11	F1	J.F. Chen et al, Euphytica, 96, 3 (1997)
Cucumis sativus x C. hystrix	0.04	0	14	F1	J.F. Chen et al, Canadian Journal of Botany, 82 (2004)
Brassica juncea x B. rapa (variety toria)	0.012	0.625	8	F2	B.R. Choudhary et al, Plant Breeding, 21, 4 (2002)
Brassica juncea x B. rapa (yellow sarson)	0.012	0.75	8	F2	B.R. Choudhary et al, Plant Breeding, 21, 4

					(2002)
Brassica rapa x Brassica napus Paper missing	0.0382	1	6	F1	L. Changming et al, Sabrao Journal of Breeding and Genetics, 33, 2 (2004)
Carica papaya x Vasconcellea quercifolia	0.156	0.2857	7	F1	R.A. Drew et al, Aust. J of Exp. Agriculture, 46 (2006)
Eucalyptus acmenoides x E. cloeziana	0.0204	0.3333	3	F1 (wild)	R.L. Stokoe et al, Annals of Botany, 88, 4 (2001)
Eucalyptus erythronema x E. stricklandii	0.0204	0.32	25	F1	K. Delaporte et al, Scientia Horticulturae, 89, 1 (2001)
Lagerstroemia indica x L. speciosa	0.049	0	5	F1	C. Pounders et al, HortScience 42, 6 (2007)
Helianthus annuus x H. debilis cucumerifolius	0.013	0.1667	11	BC	S.C. Kim, L.H. Rieseberg, Genetics, 153, 2 (1999)
Helianthus annuus x H. petiolaris	0.003	0.3333	6	BC	C. Lexer et al, Evolution, 57, 9 (2003)
Helianthus annuus x H. salicifolius	0.006	0.4118	17	F1	J. Encheva, M. Christov, HELIA, 29, 45 (2006)
Helianthus annuus x H. tuberosus	0.008	0.4707	17	F1	J. Encheva et al, HELIA, 26, 39 (2003)
Senecio vulgaris x S. squalidus	0.0327	0.5385	26	wild	A.J. Lowe et al, American jJournal of Botany, 83, 10 (1996)
Senecio vulgaris x S. squalidus	0.0327	0.5526	38	wild	J.A. Irwin, R.J. Abbott, Heredity, 69 (1992)
Lycopersicon esculentum x L. pimpinellifolium	0.0105	0.25	8	F1	A.J. Montforte et al, Theoretical and Applied Genetics, 95 (1997)
Lycopersicon esculentum x L. cheesmanii	0.0023	0.125	8	F1	A.J. Montforte et al, Theoretical and Applied Genetics, 95 (1997)
Lycopersicon esculentum x L. peruvianum	0.0291	0.1667	6	F1	S. Doganlar et al, Euphytica, 95 (1997)
Solanum melongena x S. macrocarpon	0.03	0.4737	19	F1	F. Bletsos et al, Scientia Horticulturae, 101, 1-2 (2004)
Solanum commersonii x S. tuberosum	0.073	0.5	8	F1	T. Cardi, Euphytica, 99, 1 (1998)
Solanum commersonii x S. tuberosum	0.073	0.8571	7	F1	F. Esposito et al, Journal of Agriculture and Food Chemistry, 50 (2002)
Solanum torvum x S. melongena	0.051	0	4	F1	K.R. McCammon, S. Honma, HortScience, 18, 6 (1983)
Solanum torvum x S. melongena	0.051	0.5	12	F1	F.A. Bletsos et al, Plant Breeding, 117 (1998)
Solanum acaule x S. tuberosum	0.096	0.5556	9	F1	V.-M. Rokka et al, Plant Cell Reports, 18 (1998)
Coffea liberica x C. canephora	0.0263	0.1667	12	BC	N. Amidou et al.Genetic Resources and Crop Evolution, 54, 5 (2007)
Mimulus lewisii x M. cardinalis	0.002	0.25	12	F2	H.D. Bradshaw et al, Genetics, 149, 1 (1998)
Trichostoma lanatum x T. arizonicum	0.028	0.2	5	F1	B.L. Dunn and J.T. Lindstrom,

					Hortscience, 43 (2), 2008
Trichostoma lanatum x T. purpusii	0.036	0.4	5	F1	B.L. Dunn and J.T. Lindstrom, Hortscience, 43 (2), 2008
Gilia capitata capitata x G. capitata chamissonis	0.0118	0	4	F2	E. Nagy, Evolution, 51, 5 (1997)
Dianthus giganteus x D. carthusioanorum	0.011	0.4667	15	F1	S.Y. Lee et al, Scientia Horticulturae, 105, 1 (2005)
Cerastium alpinum x C. glomeratum	0.004	0.1852	27	wild	A.R. Hagen et al, Plant Systematics and Evolution, 230, 3-4 (2002)
Amaranthus retroflexus x A. cruentus	0.0155	0	4	wild	V. Lanta et al, Plant Soil and Environment, 49, 8 (2003)
Oryza sativa x O. glaberrima	0.0105	0.4444	9	BC	G. Aluko et al, Theoretical and Applied Genetics, 109, 3 (2004)
Oryza sativa japonica x O. rufipogon	0.011	0.375	8	BC	P. Moncada et al, Theoretical and Applied Genetics, 102 (2001)
Zea mays x Z. diploperennis	0.2472	0.1818	11	F1	G. Srinivasan, J.L. Brewbaker, Maydica, 44, 4 (1999)
Hordeum vulgare x H. spontaneum	0.0078	1	5	F2	U. Vega K.J. Frey, Euphytica, 29 (1980)
Sorghum bicolor x S. macrospermum	0.132	0	4	F1	H.J. Price et al, Australian Journal of Botany, 53, 6 (2005)
Carex castanea x C. arctata	0.008	0.1538	13	F1 (wild)	M.J. Waterway, Canadian Journal of Botany, 72, 6, (1994)
Allium chinense x A. schubertii	0.285	0.4545	11	F1	Y. Nomura et al, Scientia Horticulturae, 95, 3 (2002)
Allium thunbergii x A. caeruleum	0.1615	0.6364	11	F1	Y. Nomura et al, Scientia Horticulturae, 95, 3 (2002)
Allium thunbergii x A. nutans	0.1265	0.1818	11	F1	Y. Nomura et al, Scientia Horticulturae, 95, 3 (2002)
Cypripedium candidum x C. nubescens	0.012	0.2069	29	wild	K. Klier et al, Journal of Heredity, 82, 4 (1991)
Vanilla planifolia x V. aphylla	0.159	0	5	F1	M. Divakaran et al, Scientia Horticulturae, 2006
Lilium nobilissimum x L. regale	0.0893	0.4	5	F1	Y. Obata, Scientia Horticulturae, 84, 1-2 (2000)
Piper nigrum x P. barberi	0.0906	0.6667	12	F1	B. Sasikumar et al, Journal of Horticultural Science & Biotechnology, 74, 1 (1999)
Piper nigrum x P. attenuatum	0.0906	0.3333	12	F1	B. Sasikumar et al, Journal of Horticultural Science & Biotechnology, 74, 1 (1999)
Nuphar microphylla x N. variegata	0.03	0	15	wild	D.J. Padgett, American Journal of Botany, 85, 10 (1998)

ANIMALS

Rutilus rutilus x Abramis brama	0.1233	0	5	wild	A.B. Wood, D.R. Jordan, Journal of Fish Biology, 30 (1987)
Notemigonus crysoleucas x Scardinius erythrophthalmus	0.0332	0.3421	37	F1	N.M. Burkhead, J.D. Williams, Transactions of the American Fisheries Society, 120 (1991)
Leuciscus cephalus x Chalcalburnus chalcoides	0.1271	0	38	wild	B. Ünver, F. Erk'Akan, Journal of Fish Biology, 66 (2005)
Leuciscus cephalus x Chalcalburnus chalcoides	0.1271	0.3438	32	wild	P.S. Economidis, A.I. Sinis, Journal of Fish Biology, 32 (1988)
Semotilus atromaculatus x Campostoma anomalum	0.1905	0	10	F1	M.R. Ross, T.M. Cavender, Copeia, 1981, 2 (1981)
Semotilus atromaculatus x Nocomis biguttatus	0.18	0	7	F1	M.R. Ross, T.M. Cavender, Copeia, 1981, 2 (1981)
Semotilus atromaculatus x Rhinicthys atratulus	0.196	0.4	10	F1	M.R. Ross, T.M. Cavender, Copeia, 1981, 2 (1981)
Notropis spiloperus (Cyprinella spiloptera) x N. whipplei (C. whipplei)	0.1302	0.619	21	F1	N.A. Neff, G.R. Smith, Systematic Zoology, 28, 2 (1979)
Lepomis cyanellus x L. macrochirus	0.1803	0.1765	17	F1	N.A. Neff, G.R. Smith, Systematic Zoology, 28, 2 (1979)
Pleuronectes ferrugineus x P. americanus	0.0775	0.3333	9	F1	I-S. Park et al, Aquaculture Research, 34 (2003)
Salvelinus confluentus (Oncorrhynchus tshawytsha) x S. fontinalis	0.134	0.6	10	wild	R.F. Leary et al, Systematic Zoology, 32, 4 (1983)
Cottus bairdi x C. cognatus	0.0373	0.3846	13	wild	R.E. Strauss, American Midland Naturalist, 115, 1 (1986)
Passerina cyanea x P. amoena	0.0387	0.4	5	wild	M.C. Baker, M.S. Johnson, The Auk, 115, 2 (1998)
Dendroica magnolia x D. coronata coronata	0.089	0.25	4	wild	S.C. Latta et al. The Auk, 115, 2 (1998)
Peromyscus maniculatus x P. polionotus	0.0504	1	4	F1	W.D. Dawson, Evolution, 19, 1 (1965)