Naturwissenschaften (2007) 94:667 674
DOI 10.1007/s00114 007 0245 z

ORIGINAL PAPER

The attractiveness fragment—AFLP analysis of local adaptation and sexual selection in a caeliferan grasshopper, *Chorthippus biguttulus*

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Received: 8 August 2006 / Revised: 12 March 2007 / Accepted: 16 March 2007 / Published online: 4 April 2007

Abstract Genetic variability among males is a necessary precondition for the evolution of female choice based on indirect genetic benefits. In addition to mutations and host–parasite cycles, migration of locally adapted individuals offers an explanation for the maintenance of genetic variability. In a previous study, conducting a reciprocal transplant experiment on a grasshopper, *Chorthippus biguttulus*, we found that environmental conditions significantly influenced not only body condition but also an important trait of male calling song, the amplitude of song. Although not significant, all other analysed physical and courtship song traits and attractiveness were superior in native than in transferred males. Thus, we concluded that local adaptation has a slight but consistent influence on a range of traits in our study populations, including male acoustic attractiveness. In our present study, we scanned male grasshoppers from the same two populations for amplification fragment length polymorphism (AFLP) loci connected with acoustic attractiveness to conspecific females. We found greater differences in allele frequencies between the two populations, for some loci, than are expected from a balance between drift and gene flow. These loci are potentially connected with locally adapted traits. We examined whether these alleles show the proposed genotype environment interaction by having different associations with attractiveness in the two populations. One locus was significantly related to sexual attractiveness; however, this was independent of the males' population affiliation. Future research on the evolution of female choice will benefit from knowledge of the underlying genetic architecture of male traits under intraspecific sexual selection, and the 'population genomics' approach can be a powerful tool for revealing this structure.

Keywords Sexual selection · Female choice · Local adaptation · Grasshopper · *Chorthippus biguttulus* · AFLP · Genetic variation

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Introduction

Acoustic communication plays an important role during courtship and mate choice in many animal species. Usually, males produce acoustic signals according to which females choose their mating partners (e.g. Burpee and Sakaluk 1993; Dagley et al. 1994; Gerhardt 1991; Balakrishnan et al. 2001; Marquez and Bosch 1997; Reinhold et al. 1998; Tuckerman et al. 1993; von Helversen and von Helversen 1997; Klappert and Reinhold 2002). In species in which females do not get any direct benefits from their partners, the evolution of costly choice behaviour might be explained by genetic benefits females gain by preferring certain males over others (Andersson 1994). Acoustic signals might therefore indicate a male's superior quality as a mating partner (e.g. Brown 1999; Welch et al. 1998). The fitness benefits for females may either be gained in terms of more attractive sons or by an enhanced viability of offspring (reviews in Andersson 1994; Kokko et al. 2002). Although

this topic has been studied intensively, the question of how the genetic variability in males is maintained in spite of strong directional sexual selection persists. The occurrence of detrimental mutations and the co-evolution of host–parasite cycles (Hamilton and Zuk 1982; Iwasa et al. 1991; Rowe and Houle 1996) have been suggested as potential mechanisms. A further explanation for perpetual female choice might be the immigration of maladapted individuals into locally adapted populations (Reinhold 2004; Klappert and Reinhold 2005). According to this theory, males do not differ per se in courtship traits among different populations, but they vary in performance due to genotype–environment interactions. Hence, females are not expected to mate assortatively with males from their own population but with males that perform better under the current conditions.

In any of the above-mentioned scenarios, a necessary precondition for beneficial female choice is the females' ability to identify superior males. Additionally, male quality has to have a heritable component measured in terms of attractiveness. If traits related to attractiveness are to some extent heritable, then it might be possible to identify DNA sequences in male genomes that are linked to attractiveness. Most studies use laboratory populations and inbred lines to determine associations between the courtship phenotype and the underlying quantitative trait loci (QTL) or genes (Gleason et al. 2005, 2002; Suvanto et al. 2000). It is notoriously difficult, however, to confirm that the same genes or QTL found in laboratory experiments are involved in the expression of courtship traits in wild populations with natural genetic variance and unknown pedigrees (Slate 2005). Furthermore, these studies focus on traits used in the context of interspecific mate recognition in studies of speciation (e.g. Macdonald and Goldstein 1999; Saldamando et al. 2005; Shaw and Parsons 2002), rather than dealing with the genetics underlying male quality in intraspecific mate choice.

In our study, we tested for genetic correlates of sexual attractiveness in wild males of the grasshopper species, *Chorthippus biguttulus*, using the time- and cost-efficient amplified fragment lengths polymorphism method (AFLP; Vos et al. 1995). An innovative association analysis approach allowed us to circumvent the problems of unknown pedigrees in determining regions in the genome related to courtship traits. Recordings of courtship song from wild-caught males were used in playback experiments to determine the males' overall attractiveness to females. In a previous reciprocal transplant experiment, we showed that transfer to a novel environment had a slight but consistent negative effect on all analysed male traits (Klappert and Reinhold 2005) and a significant negative effect on immunocompetence (Kurtz et al. 2002). Native males always performed better, on average 15.9%, than transferred males. A substantial reaction range amongst the traits was observed: Some traits responded very sensitively to the transfer with a maximum difference between native and transferred males of 103.8%, while other traits showed only small differences (1%, percentages obtained from non-standardised trait values). Although the two male groups did not differ significantly in their attractiveness, we were not able to falsify the hypothesis that *C. biguttulus* females preferred locally adapted native males over migrated males. A power analysis showed that a difference of about 16% between native and transferred males would have been necessary to confirm a significant difference in attractiveness between the two male groups, while we found only a difference of about 1%. Thus, we conclude that our results suggested the occurrence of local adaptation in the two study populations and a potential influence of adaptation on male attractiveness. We chose to analyse the genetic variance of overall attractiveness rather than individual courtship song elements for the current study because female preference functions in *C. biguttulus* exerted different selection pressures on different song parameters, partly in a non-linear way (Klappert and Reinhold 2002). Song traits were not different per se between the two study populations, as expected by the local adaptation hypothesis, but autochthonous and allochthonous males differed in their courtship performance (significantly so in the amplitude of song and consistently but non-significantly in all other song traits). Thus, the best way to capture the difference in performance of native and transferred males was to use attractiveness rather than individual song traits.

The first aim of the present study was to identify AFLP loci with differences in allele frequency between populations greater than expected from the balance between mutation, gene flow and genetic drift that characterises neutral variation. These 'outlier' AFLP loci are candidate markers for genomic regions involved in local adaptation (Wilding et al. 2001). We used only these AFLP fragments for further analyses because the local adaptation hypothesis predicts that females should prefer different genotypes in different environments. If female preferences are, in part, maintained by selection to prefer locally adapted males, we predict that some of these locally differentiated AFLP loci will also be associated with male attractiveness to females. We test this prediction by correlating AFLP phenotypes at outlier loci with the acoustic attractiveness of their bearers.

Materials and methods

Study species

The bow-winged grasshopper *C. biguttulus* is a univoltine grasshopper species widely distributed throughout Western Europe. Males produce a calling song by stridulation, to

which receptive females respond differentially (von Helversen 1972; von Helversen and von Helversen 1975, 1981, 1983, 1998). Females answer with their own acoustic signal if they are stimulated by an attractive calling song, leading to reciprocal singing between females and males. The male approaches the female phonotactically following her response signal, and mating is likely to occur. The attractiveness of males can be determined by performing sequential acoustic female choice experiments in which the response rates of females to playbacks of male songs are recorded. The resulting attractiveness values are good predictors for male-mating success (Klappert and Reinhold 2002). Male song traits were determined using a custom-made computer program described in detail elsewhere (Klappert and Reinhold 2002).

For the present study, we analysed the same individuals that had been used in an experiment on the influence of local adaptation on sexual selection (Klappert and Reinhold 2005). Individuals originated from two populations near Bonn in Germany. The environmental conditions differed widely between these two populations, located approximately 50 km apart. Individuals from the population "Sieg" stemmed from a meadow near the estuary of the river Sieg into the Rhine (7°6′E, 50°46′N, 60 m above sea level (ASL)) feeding mainly on soft grasses. The population "Eifel" originated from a steep south-facing glade in a mountainous forest of the Eifel area southwest of Bonn (7° 3′E, 50°23′N, about 600 m ASL). Here, the grasshoppers fed mainly on comparatively hard grass blades. From the experiments described in the earlier study, we obtained acoustic attractiveness values and song traits of the males. For further details on ecological differences between the two study sites, on the experimental regime of transfer and on details on how attractiveness values were obtained, see Klappert and Reinhold (2005). In short, freshly hatched larvae were marked according to their genetic origin and released either in their native or the foreign population. Females were recaptured as last instar larvae to ensure virginity, while males were recaptured after they reached maturity. The acoustic attractiveness of males was determined by playing back their courtship songs to virgin mature females. Songs from 26 randomly chosen native and 24 transferred males retrieved from the Eifel habitat were played back once to native (N=23) and transferred (N=29) virgin females collected there, while the song from 25 native and 25 transferred males recaptured at the Sieg were played back to native (N=28) and transferred (N=13) females collected at the Sieg. During playback, we recorded how often females responded to each song. The response rate of individual females and the overall number of responding females to a playback were used to calculate an individual attractiveness value for each male. As these values did not follow a normal distribution, attractiveness values were square-root transformed before applying parametric tests. Females did not differ in their preference for allochthonous or autochthonous males; thus, female origin was neglected in further analyses.

DNA extraction and AFLPs

We used the AFLP method (Vos et al. 1995) to sample the genome of male grasshoppers for regions connected to their acoustic attractiveness for conspecific females, an example of a 'population genomics' approach (Luikart et al. 2003). Grasshoppers were anaesthetised with CO_2 before killing them in 98% EtOH, in which they were stored. DNA was extracted from the dissected heads using a standard cetyltrimethylammonium bromide (CTAB) isolation protocol (after Doyle and Doyle, http://irc.igd.cornell.edu/Protocols/DoyleProtocol.pdf). The AFLPs analysis followed the protocol used by Wilding et al. (2001). Briefly, isolated and purified genomic DNA was double restricted with 5 U Eco*RI* and 3 U Mse*I* before ligating adaptors. A preselective amplification was performed with primers complementary to the core of the adaptor sequences. The subsequent selective polymerase chain reaction (PCR) amplification was performed with radioactively labelled Eco+4 primers so that the amplified fragments, which were separated on a polyacrylamide gel, could be visualised on autoradiographs. For primer sequences, see Table 1. It was necessary to use an extension of four selective nucleotides to obtain a manageable number of loci per primer for the extraordinarily large genome of caeliferian grasshoppers (Tatsuta and Butlin 2001). The estimated genome size of *C. biguttulus* (derived from ten closely related species taken from the Animal Genome Size Database (Gregory 2005)) is with 11.08 pg (±2.18 SD) very large, approximately 22×10^9 base pairs. This is several times larger than that of most other animal species analysed with AFLPs so far (e.g. snails *Littorina* (Wilding et al. 2001), crickets *Laupala* (Parsons and Shaw 2001), damselflies *Nehalennia* (Wong et al. 2001) or gypsy moths *Lymantria*, (Reineke et al. 1999) all with genome sizes between 2 and 4×10^9 base pairs (data taken from Gregory 2005, but see Whitlock et al. (2006) for amphibians).

Data analysis

Because of the mainly dominant nature of AFLP markers, the autoradiographs only allowed the scoring of absence or presence of a fragment. It is necessary for subsequent analyses to assume that (dominant) same-sized fragments are independent and homologous character states of one locus and that all the recessive AFLP alleles of one locus are identical in state resulting in the absence of amplification. Additional assumptions, in some analyses, are a

Table 1 Adapters, pre selective and selective primer sequences used for the AFLP analysis

Primers/adapter	Sequence
Adapters	
Eco*RI*	5′ CTCGTAGACTGCGTACC 3′ 3′ CATCTGACGCATGGTTAA 5′
Mse*I*	5′ GACGATGAGTCCTGAG 3′ 3′ TACTCAGGACTCAT 5′
Primers	
Pre amplification	
Eco+1	
Eco+C	5′ GACTGCGTACCAATTCC 3′
Mse+1	
Mse+C	5′ GATGAGTCCTGAGTAAC 3′
Selective amplification	
PCR	
Eco+4	
Eco+CAGA	5′ GACTGCGTACCAATTCCAGA 3′
Mse+4	
Mse+CGAG	5′ GATGAGTCCTGAGTAACGAG 3′
Mse+CAAT	5′ GATGAGTCCTGAGTAACAAT 3′

All PCRs were performed with a concentration of 1.5 mM $MgCl_2$; the annealing temperature for all primers is 56°C.

Mendelian segregation of fragments and that all AFLP loci are in Hardy–Weinberg equilibrium.

Autoradiographs were scored manually for the presence or absence of bands. Altogether, the genome of 99 grasshoppers with 50 males originating from the "Eifel" and 49 from the "Sieg" population were analysed. Samples of several individuals were loaded on all gels to serve as references allowing correct identification of all loci scored. Loci, which could not be identified consistently and without doubt on all autoradiographs, were omitted from further analysis. A locus was defined as polymorphic if at least one individual showed a variant pattern (see Wilding et al. 2001).

Differences in phenotype composition (frequency of a present or absent band at one locus) between the two populations were identified with *G* tests. Due to zero values in some cells of the data matrix, it was necessary to add one count to all cells for a correct analysis with the *G* test. All *G* values were corrected by William's correction, and critical *G* values were adjusted with the Dunn–Šidák method (Sokal and Rohlf 1997) for multiple testing. This purely phenotypic analysis does not make any assumptions about the mode of inheritance or Hardy–Weinberg equilibrium. Loci identified as diagnostic were further analysed with respect to their potential relationship to the attractiveness of the males. Loci were added as linear factors to an analysis of variance (ANOVA) with *attractiveness* of males as dependent variable to identify loci connected to attractiveness independent of the population affiliation of the males. A *population* × *locus* interaction gives information about whether the phenotypic state associated with attractiveness in one population is the same as in the other population.

The null hypothesis for the *G* test is equality of phenotype frequencies in the two populations. This ignores the fact that some differentiation in underlying allele frequencies is expected under a balance between mutation, gene flow and genetic drift and that this differentiation will vary among loci (e.g. Beaumont and Nichols 1996). To allow for these effects, we used the simulation method of Wilding et al. (2001), implemented in the WINKLES software (http://www.fbs.leeds.ac.uk/staff/grahame/future.php), to generate an expected distribution of differentiation, measured as F_{ST}. We assumed a mutation rate of μ=0.0001 and effective population size of 1,000. The migration rate, *m*, was adjusted to fit the observed mean F_{ST}. This allowed us to simulate 10,000 bi-allelic loci with the same overall mean F_{ST} as the observed data. As the simulated distribution of F_{ST} is dependent on mean allele frequency, we then compared the observed F_{ST} value for each locus with the simulated distribution for loci with similar mean allele frequencies (see Wilding et al. 2001 for further details).

As AFLP loci segregate as dominant markers, we assumed genotypes to be in Hardy–Weinberg equilibrium (HWE) and dominance of band-presence over the absence of a band. Allele frequencies and heterozygosities were estimated according to the method of Lynch and Milligan (1994), correcting for less accurate gene frequency estimates provided by dominant markers.

The variation of heterozygosity amongst populations and loci was analysed with ANOVA. Populations and loci were entered as factors, with loci being treated as a random effect. To estimate the genetic distance between the two populations, we used *POPGENE* (version 1.31, calculation for dominant marker and diploid data) to determine Nei's genetic diversity index G_{ST}. From G_{ST}, an estimate of gene flow (N_m) measured as the number of migrating males per generation between the two sampled populations (out of the many existing populations that exchange migrants) was calculated with

$$N_m = 0.25(1 - G_{ST})/G_{ST}.$$

Sequencing of an extracted AFLP band

One AFLP band of interest (locus 26) was excised from the polyacrylamide gel, overlaying the autoradiograph to determine the correct position. DNA was extracted using a commercial gel extraction kit (Promega) and re-amplified using the selective PCR primers. The single product was sequenced commercially (Oxford Sequencing Service).

Results

Scoring

A total of 84 unambiguously scorable loci were derived from two primer combinations. Compared with other studies, this is a relatively low yield (e.g. in periwinkles, Wilding et al. 2001; in crickets, Parsons and Shaw 2001; but see Yan et al. 1999 in mosquitoes). The low number of scorable bands was due to a high proportion of fragments, which could not be scored unequivocally because of background smears on the autoradiographs. These background fragments might be the result of mismatches in the amplification of fragments, which is more likely when primers with four instead of three selective nucleotides are used (Vos et al. 1995).

Levels of polymorphism

The two primer combinations supplied a total of 84 unambiguously scorable loci. EcoCAGA with MseCGAG provided 31 scorable bands, while EcoCAGA with MseCAAT yielded 53 bands. The overall level of polymorphism was exceptionally high: 98.8% (83 from a total of 84 loci). It differed slightly between the two populations with 94.0% in the "Sieg" and 96.4% in the "Eifel" population.

Levels of heterozygosity and gene diversity

To determine the level of expected heterozygosity H_e within the two populations, it was assumed that genotype frequencies of all loci were in HWE. $H_e \pm$SE was 0.235± 0.017 in the "Eifel" population and 0.236±0.017 in the "Sieg" population, which was statistically indistinguishable (ANOVA with populations and loci as factors, $F_{1,83}=0.001$, $P=0.978$). Heterozygosity varied significantly among the loci ($F_{83,83}=14.62$, $P<0.001$). Nei's analysis of gene diversity (Nei 1987) provided a mean G_{ST} of 0.0101± 0.0016 calculated over all loci and 98 individuals, resulting in an N_m estimate of 24.6. This suggests a high level genetic connectivity between the two populations "Sieg" and "Eifel", although they are 50 km apart and live under very different environmental conditions.

Differentiating loci for the two populations

We examined with G tests (G corrected after William's correction for an R×C table, Sokal and Rohlf 1997) whether individuals originating from the "Sieg" or the "Eifel" showed different phenotype frequencies. Overall, the two populations differed significantly ($G_{1,84}=132.22$, $P<0.005$), one locus was significantly different and one locus tended to be different in phenotype composition between the two populations after correction for multiple comparisons (Table 2). To confirm that the two populations have significantly different allele frequencies at locus 26, we considered simulated F_{ST} values obtained by the WINKLE program for mean allele frequencies between 0.7 and 0.9 (locus 26 mean allele frequency, ≈0.8). Of the 1,997 values in this category, only 10 ($P=0.005$) exceeded the observed value for locus 26 ($F_{ST}=0.085$). No other observed value fell outside the expected distribution. Locus 10 had the next highest F_{ST} (0.052), but it did not exceed expectation.

Loci differing between populations and their relationship to attractiveness

The two loci identified to possess a significant difference or a tendency to differ in allele composition in individuals originating from different populations were further analysed with respect to their potential relationship to the attractiveness of the males. An ANOVA with *attractiveness* as dependent variable and the two population diagnostic loci 26 and 10 as factors was performed. Only the interaction term population × locus 26 was included in the analysis, as locus 10 was fixed for the "presence" allele in the Eifel population. As the interaction term was non-significant (Table 3), the ANOVA was repeated without it (Engqvist 2005). This did not change the outcome of the analysis (population $F_{1,98}=0.071$, locus 10 $F_{1,98}=0.084$, both n.s., locus 26 $F_{1,98}=7.98$, $P=0.006$).

Table 2 G values for population differences in phenotype frequency (with William's correction for 2×2 tables, from Sokal and Rohlf 1997)

Primer combination	EcoCAGA MseCGAG				EcoCAGA MseCAAT		
	N=99 ("eifel" N=50, "sieg" N=49)				N=97 ("eifel" N=49, "sieg" N=48)		
Locus	**2**	**10**	**25**	**26**	**54**	**62**	**81**
G	5.03	10.85	5.15	16.17	6.20	5.41	5.68
P	<0.1	**<0.1**	<0.1	**<0.01**	<0.05	<0.1	<0.05

Alpha was corrected using Dunn Šidák method; significant results after correction are given in a bold font. For all tests $df=1$, only population diagnostic loci are presented (overall $G_{1,84}=132.22$, $P<0.005$).

Table 3 Results of ANOVA with attractiveness values of males as dependent variable and population, locus and their interaction as independent variables

	df	*F*	*P* value
Population	1	0.584	0.447
Locus 10	1	0.035	0.852
Locus 26	1	8.945	0.004
Population × locus 26	1	1.023	0.314
Error	94		
Total	98		

Only the locus identified to possess significant different allele frequencies (locus 26, $P<0.01$) in the two populations "Eifel" and "Sieg" and the locus with $P<0.1$ (locus 10) were tested. Locus 10 is fixed for the "present" allele in the "Eifel" population; thus, the interaction term can not be calculated.

The presence or absence of a band at locus 26 was found to be significantly related to the attractiveness value of males (Table 3). Males carrying a DNA fragment at this locus were more attractive than males lacking that band (Fig. 1). This difference was more pronounced in the "Sieg" population, but population affiliation had no significant influence on the direction of the relationship between attractiveness and allelic state of that locus (Table 3).

Sequencing of the "attractiveness fragment"

Bands from locus 26 were extracted from a polyacrylamide gel from two individuals, one from each of the two populations, cleaned and sequenced. The amplified DNA fragment was 256 base pairs long, and a global optimal alignment resulted in 100% identity, confirming that homologous products had been sequenced. A BLASTn search at NCBI did not identify any similarities of the DNA fragment with any known genes or anonymous sequences, and translating the sequence for all possible reading frames did not result in any known protein (BLASTp). The sequence has been deposited in the EMBL database with accession number AM261031.

Discussion

The local adaptation hypothesis predicts that some genomic regions will harbour loci that influence both local adaptation and male attractiveness. To our knowledge, the present study is the first to use individuals with natural genetic variance and an unknown pedigree to test this expectation. We succeeded in identifying one genomic region, from a small sample of regions marked by polymorphic AFLP bands, which differed significantly in allele frequency between two populations and was also linked to acoustic attractiveness in male *C. biguttulus*. Attractiveness of males carrying the "present" allele at that locus was significantly higher than that of males without a band at that locus.

Overall, fragments scored in each population showed a very low differentiation in heterozygosity, with values of 0.236 and 0.235. A low G_{ST} value, as reported here, indicates that the largest fraction of total gene diversity in the entire sample of individuals can be ascribed to diversity within populations. This clearly shows that the two populations studied in this paper have not diverged for neutral loci, as expected for populations in relatively close regional proximity.

Despite a strong genetic connectivity between the two populations, local adaptation was not only observed in a reciprocal transfer experiment (Klappert and Reinhold 2005) but also detectable in the underlying genetic structure of individuals from the two populations. The two identified loci are likely associated with locally adapted traits, and thus, we predicted that they might be related to population specific properties important for sexual attractiveness. Remarkably, one of these two loci (locus 26) was found to be associated with sexual attractiveness of male grasshoppers. However, males showing a band at that locus were significantly more attractive than males homozygous for the recessive 'absence' allele without the population × phenotype interaction predicted by the hypothesis of sexual selection based on local adaptation. One possible explanation might be that female preferences result in a selection favouring a trait linked to locus 26, which is opposed by different strengths of natural selection in the two populations.

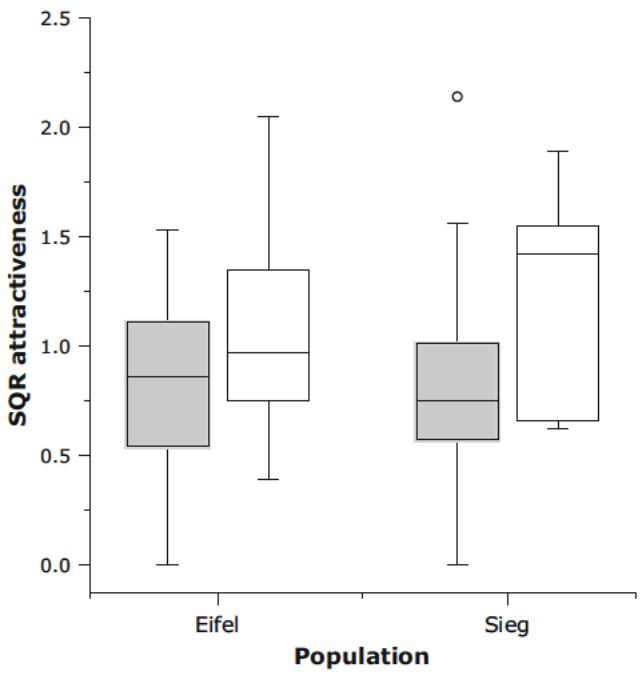

Fig. 1 Transformed attractiveness values (median±50% quartiles, extremes) for individuals from both populations, separated for males with absent (*hatched*) or present (*white*) bands at locus 26

As a next step in analysing the association between locus 26 and attractiveness, we extracted the band from a polyacrylamide gel from individuals with a fragment at that locus. The fragment was sequenced but, not surprisingly, it failed to match any known sequence or protein. Nevertheless, this sequence might make it possible to develop a co-dominant marker at the locus for future studies and to investigate neighbouring sequences for evidence of selection. The next step in a follow-up study would thus be to sequence more individuals from different populations for the fragment to analyse the degree of polymorphism. Additionally, to confirm the association between acoustic attractiveness and the attractiveness fragment, males from different populations should be analysed with respect to acoustic attractiveness for females and subsequently genotyped.

Our result of one locus associated with sexual attractiveness of males is a conservative estimation because AFLP markers cannot reliably be used to differentiate between fragments present in one or two copies, i.e. whether occurring in heterozygous or homozygous condition. This inability to distinguish all genotypes reduces the power to detect associations between AFLP loci and loci that influence attractiveness. In any case, our survey of 84 loci represents a tiny fraction of the genome. Much larger surveys are now becoming possible using a variety of markers (Luikart et al. 2003; Butlin and Roper 2005). Our study demonstrates the potential for such screens to test the predicted association between local adaptation and sexual selection by female preference.

In recent times, studies have become available on the genetic basis of traits influenced by female preference. However, most of these studies concentrated on traits involved in sexual isolation among species and employ classical methods like backcross experiments (e.g. Doi et al. 2001) or molecular genetic methods like quantitative trait locus (QTL), e.g. in crickets *Laupala* (Shaw and Parsons 2002), *Drosophila* (Macdonald and Goldstein 1999; Zeng et al. 2000) or mosquitoes *Aedes* (Yan et al. 1999). One of the most interesting questions in evolutionary biology at presence, although, asks whether loci that show genetic variation amongst populations or species are the same that vary within populations and whether the same genetic architecture underlies species isolation and sexual selection. Thus, it is necessary to test the importance of classically identified candidate loci for sexually selected traits within natural populations. However, our present study shows that it is also possible to go the other way: Loci associated with sexual selection within natural populations can be identified first and then examined further, e.g. by comparing them to known sequences and proteins. With the rapidly increasing availability of completely sequenced and annotated organisms, this approach might prove a valuable tool for behaviour genetics.

Our result suggests that combining genome scans with measures of important phenotypes like male attractiveness will be a productive approach to the genetic analysis of sexual selection.

Acknowledgements We would like to thank Haruki Tatsuta for indispensable help with lab work as well as for statistical inspiration. All people working at lab 10.08 in the School of Biology at Leeds University were always a source of fruitful discussion and helped wherever they could. The authors would like to thank Anne Seelbach, Carsten Pollmann, Robert Jehle, Jens Krobbach and three anonymous referees for useful comments on the manuscript. The present study was financially supported by the Deutsche Forschungsgemeinschaft (RE 1167/3) and by the EU with a Marie Curie EST Grant. All experiments conducted comply with the current laws of Germany.

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