

Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*

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In *Drosophila montana*, male courtship song frequency is closely associated with male courtship success and offspring survival. Other pulse characters (pulse length and cycle number) may also affect female mate choice, whereas pulse train characters (interpulse interval, pulse number and pulse train length) are not associated with these male fitness components. Inbreeding depression in these song characters was investigated by comparing the songs of inbred and outbred fly strains. The average change in most song characters as a result of inbreeding was only a few percent. However, in male song frequency the average inbreeding depression was about 14%, suggesting that this song character is associated with fitness. Outbreeding depression and the genetic architecture of song characters were investigated with interpopulation crosses and joint scaling tests. For pulse train characters the generation means show only evidence of additivity, and the existence of dominance or epistasis in these characters was strongly rejected in each case. In pulse characters the means of the F_1 males were lower than the average of the parental generations. In pulse length and cycle number this difference was attributable to dominance alone. In frequency there was outbreeding depression also in the F_2 generation, suggesting a break-up of favourable epistatic gene combinations. The outbreeding depression in this character in the F_1 generation was caused by dominance, and in the F_2 also by duplicate epistasis between dominant decreaseers. The possible role of outbreeding depression and epistasis in speciation is discussed.

Keywords: courtship song, epistasis, interpopulation hybrids, shifting balance theory.

Introduction

The most striking observed consequence of inbreeding is the reduction of the mean phenotypic value shown by characters connected with fitness, the phenomenon known as inbreeding depression (Falconer, 1985; Thornhill, 1993). Under inbreeding, primary fitness characters tend to exhibit very high levels of inbreeding depression, whereas characters more remotely related to fitness change normally show only low levels (Lynch & Walsh, 1998; De Rose & Roff, 1999). On the other hand, extreme outcrossing may also decrease offspring fitness; for example, in crosses between distantly related populations, F_1 and/or F_2 generations are sometimes less fit than the average of the original parental strains (Wu & Palopoli, 1994; Lynch & Walsh, 1998). This phenomenon is known as outbreeding depression, and it is

usually attributed to a break-up of favourable epistatic interactions in the parental lines, or phenotype–environment interaction (Thornhill, 1993; Lynch & Walsh, 1998; Wade & Goodnight, 1998).

In *Drosophila montana*, some courtship song characters are associated with male fitness. Courtship song in this species consists of a train of polycyclic sound pulses (Fig. 1). In the wild, females prefer males that produce short and dense sound pulses, a combination which leads to a high carrier frequency (Aspi & Hoikkala, 1995). Pulse train characters, i.e. the interpulse interval, length of a pulse train and number of pulses in a pulse train, do not seem to affect female choice (Aspi & Hoikkala, 1995). These results have been confirmed by Ritchie *et al.* (1998) under laboratory conditions by using simulated background songs.

Male courtship song frequency is also associated with another fitness component. It is correlated with the survival rate of the male's progeny from egg to adulthood (Hoikkala *et al.*, 1998). Accordingly, male song frequency can act as a reliable indicator of male

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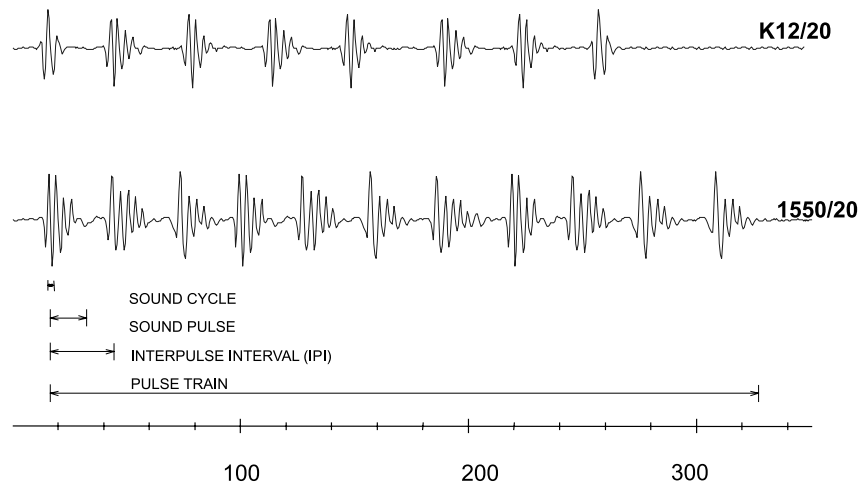


Fig. 1 Oscillograms of male courtship songs in inbred strains K12/20 and 1550/20 of *Drosophila montana*.

fitness for females. By choosing a male with courtship song frequency one standard deviation higher than the average in the population, a female could increase the viability of her offspring by about 24% (Hoikkala *et al.*, 1998). The other song characters are not associated with offspring survival (Hoikkala *et al.*, 1998).

Even though the consequences of inbreeding have been studied in several fitness-related traits (Lynch & Walsh, 1998; De Rose & Roff, 1999), there have been relatively few studies designed to investigate the effects of inbreeding on behavioural traits (but see Miller *et al.*, 1993). Data on outbreeding depression in interpopulation hybrids, especially in animals, are also sparse (Thornhill, 1993). The aim of this study is to investigate possible inbreeding depression and outbreeding depression in fitness-related and non-fitness-related male courtship song characters in *D. montana*. We would expect to find inbreeding depression in pulse characters, especially in male courtship song frequency, whereas there should not be changes in the means of pulse train characters. *Drosophila montana* also provides a good opportunity to study possible outbreeding depression. This species has a very wide circum-Arctic distribution range (Throckmorton, 1982), and thus it is possible that crosses between distant allopatric populations could lead to a significant outbreeding depression in some male song characters.

Materials and methods

Flies and crosses

The inbred strains of *D. montana* used have been inbred for 20 generations by brother–sister matings to render the strains homozygous. The strains originated from different parts of the species' distribution range. The strain K12/20 is from Kemi, northern Finland (65°40'N, 23°35'E) and the strain 1251/20 from Oulanka, north-

eastern Finland (66°25'N, 29°0'E). The strain 1550/20 is from Yukon, Alaska (61°30'N, 159°20'W) and the strain 1263/20 from Kawasaki, Japan (34°80'N, 139°60'E). The absence of genetic variation in strains K12/20 and 1550/20 has been tested by using RAPD-markers. No variation within strains was found by using 90 RAPD-markers even though 20 of these markers appeared to differ between strains (J. Aspi, unpubl. obs.).

As outbred flies I used the males of two laboratory strains, 1251 and 1263, from which the inbred lines 1251/20 and 1263/20 originated (the original laboratory strain of inbred 1550 strain was no longer available). I also used F₁ male offspring of wild-caught males from a natural population near the town of Kemi (from where the strain K12/20 originated). F₁ males were used instead of wild-caught males because songs of *D. montana* males raised under laboratory conditions seem to differ from those of wild-caught males (Aspi & Hoikkala, 1993). These differences are caused by larger phenotypic variability of song characters in the field and also by genotype–environment interactions (Aspi & Hoikkala, 1993).

As the inbred strains K12/20 and 1550/20 strains were the most dissimilar ones with respect to male song characters, and were from the opposite ends of the distribution range, I used these strains to study possible outbreeding depression or heterosis. There was a significant difference in each male song character between these strains (see Table 2). Parental, and also F₁, F₂ and backcross generations between parental strains, including all reciprocals, were raised under similar conditions.

Flies were maintained in culture bottles containing malt medium, in continuous light at 19°C. These conditions approximate conditions in the wild when the flies mate at northern latitudes. Freshly emerged flies were sexed under light CO₂ anaesthesia and maintained in food vials. Flies were used in experiments at the age of 3 weeks old, when sexually mature.

Song recording and analysis

The courtship song of *D. montana* comprises polycyclic sound pulses (Fig. 1). The song of each male was recorded with a Sony TC-FX33 cassette recorder and a JVC-condenser microphone. Song analysis was carried out using the Signal Sound Analysis System (© Engineering Design). I measured the lengths of the pulse trains (PTL) and counted the number of pulses per train (PN) in oscillograms made of the songs. I also counted the number of cycles (CN) in the fourth sound pulse of each pulse train, and measured the length of this pulse (pulse length, PL) and the distance from the beginning of this pulse to the beginning of the next one (interpulse interval, IPI). Carrier frequency of the song (FRE) was measured from Fourier spectra. For statistical analysis, PL, FRE, IPI and PTL were transformed to natural logarithms.

Only one pulse train per male was analysed because the repeatabilities of most of the song characters have been shown to be high. Aspi & Hoikkala (1993) studied the repeatability of these characters by recording the songs of males on three subsequent days. The repeatability analysis included the three *D. montana* strains used in this study (1251, 1263, 1550) and five males per strain were analysed. Repeatabilities of the pulse characters were rather high (PL = 0.85, CN = 0.85, FRE = 0.45), whereas those for pulse train characters were, on average, somewhat lower (IPI = 0.79, PTL = 0.24, PL = 0.26).

Statistical methods

The shift in the mean phenotype of song characters because of inbreeding was measured as:

$$I.D. = 1 - (\bar{z}_1 - \bar{z}_0), \tag{1}$$

where \bar{z}_1 and \bar{z}_0 are the means of the random mating base and the inbred populations (Falconer, 1985; Lynch & Walsh, 1998).

The means of different generations were analysed with a joint scaling test by using the weighted least squares method (Mather & Jinks, 1982; Kearsley & Pooni, 1996; Lynch & Walsh, 1998). This technique was used to derive estimates of midparent (*m*), additive (*[a]*), dominance (*[d]*) and additive × additive (*[aa]*), additive × dominance (*[ad]*), dominance × dominance (*[dd]*) effects for male song characters. The weighted least-squares model that incorporates additive, dominance and digenic epistatic effects is (Mather & Jinks, 1982; Kearsley & Pooni, 1996):

$$\mathbf{X} = (\mathbf{C}'\mathbf{W}\mathbf{C})^{-1}(\mathbf{C}'\mathbf{W}\mathbf{Y}), \tag{2}$$

where \mathbf{X} is the vector of mean, additive, dominance and digenic epistatic parameters, *m*, *[a]*, *[d]*, *[aa]*, *[ad]* and *[dd]*, respectively. \mathbf{C} is the matrix of coefficients for these parameters from the equation for predicted family means, \mathbf{W} is the diagonal matrix of weights (i.e. the reciprocals of the variances of the generation means), and \mathbf{Y} is the vector of generation means. The variances of the parameter estimates can be obtained from the diagonal elements of $(\mathbf{C}'\mathbf{W}\mathbf{C})^{-1}$ given that the model is adequate (i.e. the chi-squared statistic is not significant). The observed generation means were first tested for fit to models incorporating only *m*, *m* and *[a]*, and *m*, *[a]* and *[d]*, successively. The expected means of the six generations were calculated using the parameter estimates, the goodness-of-fit of the observed generation means was tested with the chi-squared statistic, and the significance of each parameter was tested by using a *t*-test (Mather & Jinks, 1982; Kearsley & Pooni, 1996). The more complex model was applied only if the chi-squared test revealed that the simpler model was inadequate.

Rejection of the additive–dominance model indicates that epistasis and/or linkage are contributing to the genetic divergence of the populations. If there is significant additivity and dominance in a character, then the full digenic interaction model incorporates six parameters, *m*, *[a]*, *[d]*, *[aa]*, *[ad]* and *[dd]*. Because the number of parameters equals the number of generation means, the adequacy of the full six-parameter model could not be tested. Accordingly, the significance of each epistatic parameter *[aa]*, *[ad]* and *[dd]* was first tested by a *t*-test (Kearsley & Pooni, 1996, p. 232), and whenever a parameter proved not to be significant only the remaining parameters were estimated from the vector \mathbf{X} after the appropriate genetic model was fitted.

The weighted least squares method was also used to interpret the variances of song characters in the different generations, and to estimate environmental variation within families (V_E), additive (V_A) and dominant (V_D) genetic components of variance, and the cross-product of the additive and genetic effects of genes that are segregating in the cross (V_{AD}). Because the theoretical variances of observed variances are not known, I used $d.f./[2(s^2)^2]$ as the initial weight in an iterative process following Hayman (1960). The parameter estimates were then used to calculate expected variances, and these were used to calculate new weights for a second iteration. The process was iterated until the estimates of parameters stabilized. First I fitted a V_A , V_D , V_{AD} and V_E model to six variances to test whether a single V_E fitted the variances of the K12/20, 1550/20 and F_1 generations (Kearsley & Pooni, 1996). If all the nonsegregating generation variances appeared to be heterogeneous, then I replaced V_E with three separate parameters, V_{E1} , V_{E2} and V_{E3} . On the other hand, if the variance of the F_1

only was different from those of the parental generations, then I replaced V_{E1} and V_{E2} with the pooled estimate V_{E12} . I then proceeded with the model fitting with V_E (or the separate parameters) and different combinations of the other variance parameters (i.e. V_E ; V_E and V_A ; V_E , V_A and V_{AD}).

Results

Inbreeding depression in male song characters

The randomly breeding strain 1251 has been kept in the laboratory since 1981 and strain 1263 since 1969. Thus it is possible that loss of genetic variation had already occurred in these strains through genetic drift. However, in most cases there was less phenotypic variation in male song characters in the inbred strains (Table 1) than in the outbred ones, suggesting that there was still some genetic variation left in male song characters in these strains.

Most song characters did not exhibit significant shifts in the mean phenotype with inbreeding in any of the inbred strains. In pulse train characters (PTL, PN and IPI) the magnitude and sign of the shift in the separate strains varied, as expected because of random drift. On the other hand, the sign of the shifts was more constant in pulse characters, even though the changes were not significant in CN and PL. The average change in most song characters was by only a few percent, suggesting that these song characters were not associated with fitness. However, in male song frequency the change was more than 8% in each strain, the average change being about 14%. Fisher's method (e.g. Sokal & Rohlf, 1969,

p. 621) was used to combine probabilities of tests between different inbred and random-breeding strains. Frequency was the only character in which the combined probability of the shift in the mean was significant (Table 1).

Analysis of generation means and variances in male courtship song characters in crosses between inbred strains of *D. montana*

The contributions of maternal or cytoplasmic effects to the differences between population means were assessed by comparing the means of reciprocal F_1 crosses. These means did not suggest any deviation from an autosomal mode of inheritance in any of the song characters (t -test; $P < 0.05$ in each case after sequential Bonferroni correction). However, nonautosomal effects could be caused by X-chromosomal, Y-chromosomal, or non-chromosomal permanent cytoplasmic or transient maternal factors which may counteract or overlap each other (e.g. De Belle & Sokolowski, 1987). Therefore I examined more thoroughly possible Y-chromosomal, transient maternal and permanent cytoplasmic factors by comparing different reciprocal F_2 s and backcrosses. There are six possible comparisons which have been described in de Belle & Sokolowski (1987). No significant differences in male song characters were found between reciprocals in any of the comparisons. Because comparisons revealed the significance of only chromosomal components, the reciprocal crosses were pooled for analysis by the joint scaling test.

Pulse train characters (IPI, PTL, PN) expressed neither outbreeding depression nor heterosis (Table 2).

Table 1 Means and standard deviations of male song characters of inbred and random-breeding strains (or F_1 offspring of wild-caught males) of *Drosophila montana*. I.D. is the relative shift in the means of male song characters because of inbreeding

	<i>N</i>	PL	CN	FRE	IPI	PN	PTL
K12/20, inbred	80	14.5 ± 2.4	4.1 ± 0.8	261.6 ± 20.5	30.9 ± 2.6	8.2 ± 0.7	267.6 ± 32.8
Kemi, wild	43	13.9 ± 2.2	4.2 ± 0.9	298.0 ± 39.1	26.6 ± 2.0	8.7 ± 0.7	255.3 ± 28.0
I.D.		-0.041	0.022	0.122**	0.159**	0.054**	-0.048
1251/20, inbred	10	21.3 ± 2.4	5.1 ± 0.6	229.4 ± 16.1	34.8 ± 1.0	9.1 ± 0.7	321.6 ± 29.8
1251, outbred	10	21.0 ± 2.4	5.2 ± 0.4	250.7 ± 31.6	37.0 ± 5.4	9.3 ± 0.5	351.9 ± 73.1
I.D.		-0.014	0.019	0.085	0.060	0.022	0.086
1263/20, inbred	10	22.4 ± 2.6	4.3 ± 0.49	203.7 ± 15.6	34.8 ± 1.8	9.5 ± 1.1	316.5 ± 32.6
1263, outbred	10	21.5 ± 2.8	4.7 ± 0.49	233.0 ± 14.6	34.0 ± 4.8	9.4 ± 1.8	333.9 ± 71.2
I.D.		-0.042	0.085	0.126**	-0.024	-0.011	0.052
Average I.D.		-0.032	0.077	0.136***†	0.081	0.022	0.030

Mann-Whitney U -test, $P < 0.01$ between inbred and outbred strains after sequential Bonferroni correction. *†Combined probabilities from independent tests of significance using Fisher's method.

PL, pulse length; CN, number of cycles; FRE, frequency; IPI, interpulse interval; PN, number of pulses per train; PTL, length of the pulse trains.

Table 2 Means (\pm SD) of the male song characters in different generations in crosses between inbred strains K12 and 1550 of *Drosophila montana*

Generation	<i>N</i>	PL	CN	FRE	IPI	PN	PTL
K12/20	43	14.5 \pm 2.4	4.1 \pm 0.8	261.6 \pm 20.5	30.9 \pm 2.6	8.2 \pm 0.7	267.6 \pm 32.8
1550/20	35	16.4 \pm 2.9	4.9 \pm 0.8	307.5 \pm 22.5	27.7 \pm 2.5	10.5 \pm 1.1	309.7 \pm 40.8
F ₁	86	14.7 \pm 1.6	4.1 \pm 0.5	271.9 \pm 22.3	29.3 \pm 2.7	9.5 \pm 0.8	293.5 \pm 28.4
F ₂	187	15.4 \pm 3.0	4.3 \pm 0.9	268.4 \pm 29.8	29.3 \pm 3.9	9.5 \pm 1.2	295.1 \pm 45.2
BC _{K12}	42	14.6 \pm 2.2	4.1 \pm 0.7	262.1 \pm 23.6	30.0 \pm 2.5	8.7 \pm 0.9	275.5 \pm 38.4
BC ₁₅₅₀	51	15.9 \pm 2.2	4.4 \pm 0.7	275.2 \pm 25.2	29.0 \pm 2.9	9.6 \pm 1.2	299.2 \pm 44.8

PL, pulse length; CN, number of cycles; FRE, frequency; IPI, interpulse interval; PN, number of pulses per train; PTL, length of the pulse trains.

The generation means show evidence only of additivity, and existence of dominance or epistasis in these characters was strongly rejected in each case (Table 3, Fig. 2d,e and f).

On the contrary, in each pulse character (CN, PL and FRE) the mean of F₁ males was lower than the average of the parental generations, suggesting outbreeding depression in CN and FRE and heterosis in PL. However, in the F₂ generation there was neither apparent outbreeding depression nor heterosis in CN and PL. The means of the F₂ generations were intermediate to those of the F₁ and the averages of the parental generations, suggesting that this difference resulted from dominance alone (Table 2), and that there were no coadapted gene complexes involved (Lynch, 1991; Lynch & Walsh, 1998). Accordingly, joint scaling tests revealed significant dominance but no epistasis in these pulse characters (Table 3). In both cases the potency ratio ($[d]/[a]$) was negative, indicating that the K12/20 parental strain has more dominant alleles, and was thus more potent in the cross. Dominance in CN was towards a lower number of pulses and in PL towards shorter pulses.

In FRE the performance of F₂ males was not intermediate to that of F₁ and the average of parental generations, which suggests a break-up of favourable epistatic gene combinations. Accordingly, FRE was the only song character which did not obey the additive–dominance model (Fig. 2c; $\chi^2_3 = 19.121$, $P = 0.0003$). This trait expressed significant dominance and also dominance \times dominance epistasis components in generation means. Dominance was towards lower carrier frequency, and the potency ratio was again negative, indicating that the K12/20 parental strain has more dominant alleles. The sign of the epistasis component suggested predominantly duplicate epistasis between dominant decreaseers.

The deviation of the line means from additivity seems not to be a result of a scaling effect in any of the pulse characters. Neither the log₁₀ nor a ‘variance stabilizing’ transformation suggested by Wright (1968) resulted in an adequate fit in any case.

In the analysis of generation variances for PN the variances of all nonsegregating generations (K12/20, 1550/20 and F₁) appeared to be heterogeneous and V_E was replaced by three separate parameters, V_{E1} , V_{E2} and V_{E3} (Fig. 2e). For CN, PL and PTL the variances of F₁ generation were different from those of the parental generations. Thus V_{E1} and V_{E2} were replaced by the pooled estimate V_{E12} (Fig. 2a,b and f).

There was only a significant additive variance component in each of the male courtship song characters (Fig. 2, Table 4). Even though the generation means suggested significant dominance in pulse song characters there was no significant dominance variance in either of these characters.

Discussion

Previously there have been relatively few studies designed to investigate the effects of inbreeding on behavioural traits. However, Miller *et al.* (1993) have evaluated effects of inbreeding for male mating ability and courtship behaviour in *D. melanogaster*. All their lines made isogenic for chromosome 2 showed significant reductions in overall mating ability, and males from all of these lines displayed impaired mating behaviour. In the present study, the only male courtship song character in which there was a significant shift in the means between random breeding and inbred lines was courtship song frequency. This result is in accordance with previous investigations with *Drosophila* in which primary fitness characters (such as viability, fertility and egg production) tend to exhibit very high levels of inbreeding depression, on average about 50% (Lynch & Walsh, 1998). The average change in FRE (14%) was smaller than for primary fitness characters, but still significant, suggesting that FRE may not itself cause variation in fitness, but reflects the variation because of genetic correlation with directly selected characters.

It is widely accepted that inbreeding depression is a consequence of dominance (Thornhill, 1993). Inbreeding in a population decreases the frequency of

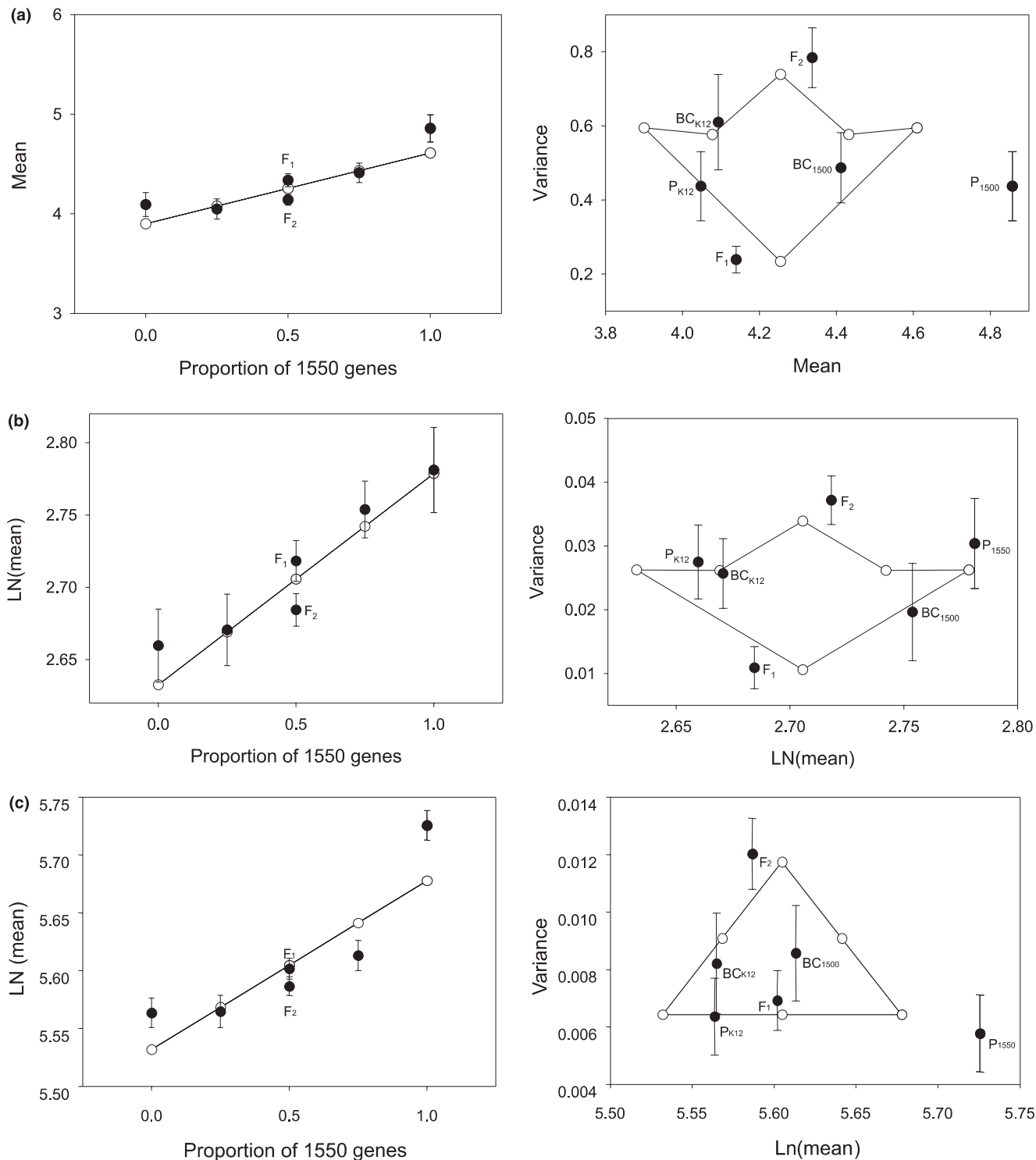


Fig. 2 Observed means and variances (\pm SE) of six courtship song characters in *Drosophila montana* (●) in relation to the predictions of the additive model. The predictions are given by the open circles on the lines on the lefthand graphs, and on the open circles on the triangles (or polygons if the environmental variance was not equal in the parental and F₁ generations) on the righthand graphs. (a) Number of cycles (CN); (b) pulse length (PL); (c) frequency FRE; (d) interpulse interval (IPI); (e) number of pulses per train (PN); (f) length of the pulse trains (PTL).

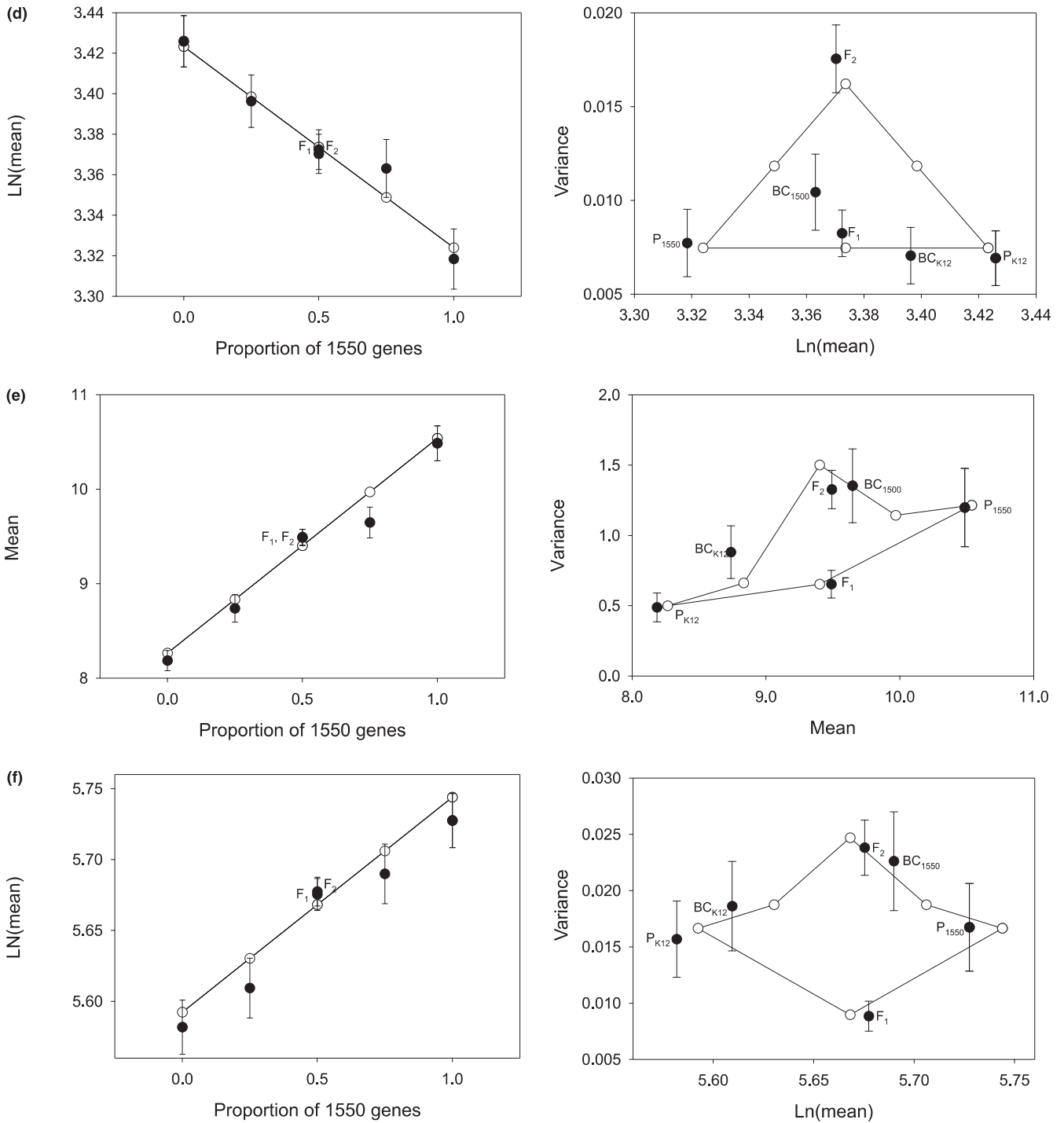


Fig. 2 (Continued)

heterozygotes, and, given that there is some directional dominance in the characters considered, this decline in heterozygotes will also cause the mean to change. In a heterotic trait, this results in inbreeding depression (Kearsey & Pooni, 1996). As expected, a significant dominance effect in generation means was found in each

pulse character (PL, CN and FRE), but not in pulse train characters (PN, PTL). Even though there was dominance in the means of pulse characters there appeared to be no significant dominance variance in either of these characters. This could suggest that the observed dominance in pulse characters is weak

Table 3 Estimates for mean [m], and additive [a], dominance [d] and dominance \times dominance interaction [dd] components of the generation means for male courtship characters of *Drosophila montana*. Only significant effects are given and the resulting models provide an adequate fit to data in all cases

Character	$m \pm SE$	$[a] \pm SE$	$[d] \pm SE$	$[dd] \pm SE$	χ^2	d.f.
PL	2.724 \pm 0.011***	0.060 \pm 0.012***	-0.034 \pm 0.017*		1.83	3
CN	4.459 \pm 0.070***	0.374 \pm 0.076***	-0.324 \pm 0.098***		1.32	3
FRE	5.646 \pm 0.009***	0.075 \pm 0.008***	-0.186 \pm 0.037***	0.144 \pm 0.035***	2.42	2
IPI	3.374 \pm 0.005***	0.050 \pm 0.009***			1.35	4
PN	9.402 \pm 0.047***	1.136 \pm 0.083***			7.06	4
PTL	5.668 \pm 0.006***	0.076 \pm 0.013***			3.68	4

* $P < 0.05$, *** $P < 0.001$; t -tests.

PL, pulse length; CN, number of cycles; FRE, frequency; IPI, interpulse interval; PN, number of pulses per train; PTL, length of the pulse trains.

(Kearsey & Pooni, 1996). On the other hand, the scaling tests based on variances are much less powerful than those based on means (Kearsey & Pooni, 1996). Thus, it is possible that lack of dominance variance resulted from insufficient power of the tests.

In addition to dominance, epistasis can contribute to inbreeding depression (e.g. Lynch & Walsh, 1998). Charlesworth (1998) has recently stated that the magnitude of inbreeding depression in *Drosophila* is too large to be accounted for by a mutational load, and one possible explanation for this discrepancy is synergistic epistasis among the fitness effects of deleterious mutations. Accordingly, it is possible that inbreeding depression in FRE could be partly caused by the observed epistasis. However, on the basis of only inbred and outbred strains, the possible contribution of epistasis to inbreeding depression could not be estimated.

Significant additive genetic variation was found in each male courtship song character, even in FRE. This seems to be in disagreement with selection theory, which suggests that there should be no additive genetic variation in characters closely associated with fitness (Lynch & Walsh, 1998). It is possible that the observed epistasis in FRE could contribute to its additive genetic variance (Chevrud & Routman, 1996a,b). Another possibility is that inbreeding in the inbred lines could have converted dominance variance to additive variance by increasing homozygosity and exposing deleterious recessive alleles to purifying selection (Willis & Orr, 1993). However, Aspi & Hoikkala (1993) have previously estimated the amount of additive genetic variance in male song characters in a natural population of *D. montana*. They have shown that the heritabilities of male song characters under laboratory conditions are high and in most cases significant. However, they also showed that heritabilities estimated across environments (estimated on the basis of wild-caught fathers and their laboratory-reared sons) were in most cases much smaller and nonsignificant, suggesting that there might not be

additive genetic variance in these characters in the wild. The low across-environment heritabilities appeared to result from larger phenotypic variability of song characters in the field and in some cases also from genotype-environment interactions (Aspi & Hoikkala, 1993).

There appeared to be significant outbreeding depression in male courtship song frequency in both F_1 and F_2 generations. Hybridization between strains from different geographical locations appeared to lead to the production of individuals that have lower fitness than either parental type. Both dominance and epistasis can have effects on the expression of mean phenotypes in male courtship song frequency of the F_1 and F_2 generations (Lynch, 1991; Lynch & Walsh, 1998). The difference between the mean in the F_1 and the average of parental generations could result from both dominance and additive \times additive epistasis. Because no additive \times additive epistasis was found in the joint scaling test, outbreeding depression in the F_1 generation seems to be caused by dominance alone. In the F_2 generation both additive \times additive and dominance \times dominance epistasis could contribute to outbreeding depression. No additive \times additive epistasis could be found in song frequency in joint scaling test, and thus the observed outbreeding depression is mainly caused by the break-up of favourable dominance \times dominance effects.

On the basis of *Drosophila* developmental genetics Moreno (1994) has recently suggested that interactions within sets of functionally related loci may be common and they can hide a potential for strong phenotypic effects. However, data on intraspecific epistatic interactions in fitness characters in animals are sparse (Thornhill, 1993). Hemmat & Eggleston (1990) have found additive \times dominance and dominance \times dominance nonallelic interactions in competitive ability of *D. melanogaster*. Hard *et al.* (1992) have determined the genetic components of population divergence in the critical photoperiod in a mosquito species. They found that in crosses between southern

Table 4 Estimates for environmental (V_E) and additive (V_A) components of the generation variances for male courtship song characters in *Drosophila montana*. Only significant effects are given and the resulting models provide an adequate fit to data in all cases

Character	$V_E \pm SE$	$V_{E1} \pm SE$	$V_{E2} \pm SE$	$V_{E3} \pm SE$	$V_{E12} \pm SE$	$V_A \pm SE$	χ^2	d.f.
PL				0.011 ± 0.002***	0.027 ± 0.004***	0.017 ± 0.002***	2.50	3
CN				0.234 ± 0.036***	0.594 ± 0.093***	0.325 ± 0.091***	2.36	3
FRE	0.006 ± 0.001***					0.005 ± 0.001***	0.74	4
IPI	0.007 ± 0.001***					0.008 ± 0.003**	1.35	4
PN		1.214 ± 0.238***	0.500 ± 0.117***	0.653 ± 0.100***		0.746 ± 0.166***	4.36	2
PTL				0.009 ± 0.001***	0.017 ± 0.003***	0.012 ± 0.003***	1.26	3

* $P < 0.05$, *** $P < 0.001$; t -tests.
 PL, pulse length; CN, number of cycles; FRE, frequency; IPI, interpulse interval; PN, number of pulses per train; PTL, length of the pulse trains.

and northern populations, epistatic effects were highly significant, indicating that genetic divergence cannot have resulted solely from differences in additively acting loci. Blows (1994) has observed also a break-up of adaptive gene complexes in fitness characters in distant populations of *D. serrata*.

Nonadditive gene interaction or epistasis is a central component of Wright's shifting balance hypotheses that postulate a creative role for small populations and founder events in character evolution and speciation (Wright, 1977). The importance of epistasis is especially true for fitness-related characters (Wright, 1969). However, both Wright's shifting balance theory and Fisher's 'large population size' theory predict lowered mean fitness with increasing local adaptation in allopatry (Wade & Goodnight, 1998). In the large population size theory, outbreeding depression occurs because of phenotype-environment interaction. Hybrids between populations are of intermediate phenotype and are not as fit as parental phenotypes in either habitat. In Wright's shifting balance theory the mean fitness of interpopulation hybrids is lower irrespective of environment because of the epistatic nature of the genetic architecture (Wade & Goodnight, 1998). Because in *D. montana* there appeared to be significant outbreeding depression in male courtship song frequency in both F₁ and F₂ generations, and the lower fitness of interpopulation hybrids was caused by nonadditive gene interaction, the present results support Wright's shifting balance theory.

In the *D. virilis* group, the male courtship songs play an important role both in species-recognition (Liimatainen & Hoikkala, 1998) and in sexual selection within the species in the wild (Aspi & Hoikkala, 1995). Thus, the epistatic nature of genetic architecture in courtship song frequency in *D. montana* could possibly lead to speciation through lowered fitness of interpopulation hybrids. However, the shifting balance theory and its role in speciation in general is still controversial (Moreno, 1994; Gavrillets, 1996; Coyne *et al.*, 1997; Wade & Goodnight, 1998).

Acknowledgements

J. O. Liimatainen, A. Hoikkala and two anonymous referees made useful comments on a previous version of this manuscript.

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