Courtship signals and mate choice of the flies of inbred *Drosophila montana* strains

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Abstract

We studied genetic variation in fly mating signals and mate choice in crosses within and between inbred strains of *Drosophila montana*. Male songs and the cuticular hydrocarbons of both sexes as well as some of the flies' behavioural traits differed significantly between strains. This did not, however, cause sexual isolation between strains. In fact, courtship was shorter if the female was courted by a male of a foreign strain than when courted by their own male. Heterosis was found for courtship duration and the carrier frequency of male song. Diallel analysis of male song revealed additive genetic variation in four out of the five traits studied. Two traits showed dominance variation and one of these, carrier frequency, expressed unidirectional dominance with alleles for higher carrier frequency being dominant. Direction of dominance in carrier frequency was the same as the direction of sexual selection exercised by *D. montana* females on this trait, which suggests that sexual selection could be a driving force in the evolution of song towards a higher carrier frequency.

Introduction

Many *Drosophila* species exhibit genetic variation in traits affecting mate choice (Scott & Richmond, 1988; Marín, 1994; Aspi & Hoikkala, 1995). This variation forms the basis for sexual selection (exercised) by the females within a species and may also give rise to isolation between geographically separated populations (Markow, 1991). Studies on genetic variation in traits affecting mate choice and progeny production are thus essential for understanding how species-specific courtship behaviour has evolved.

Mating success of the flies of different *Drosophila* species depends on a variety of factors including male courtship vigour, female receptivity, male size and the signals emitted during the courtship (studies reviewed by Spiess, 1987). A diallel crossing design (a set of n^2 possible crosses and selves) between homozygous lines makes it possible to study the effects of trait variation between strains on fly mating success.

Our study species, *Drosophila montana* Stone, Griffen, Patterson, belongs to the *D. virilis* group. It is distributed throughout the northern hemisphere, having populations in Fennoscandia, North America, Japan and Russia (Throckmorton, 1982). *D. montana* flies overwinter as adults and mate in spring (Aspi *et al*., 1993). Songs produced by males vibrating their wings are essential for stimulating the females to mate (Liimatainen *et al*., 1992). Males that have overwintered vary in their song characters due to both genetic and environmental factors (Aspi & Hoikkala, 1993; Hoikkala & Isoherranen, 1997), enabling the females to use male courtship song in their mate choice (Aspi & Hoikkala, 1995). Bartelt *et al*. (1986) have reported that *D. montana* males and females have similar hydrocarbon profiles, but whether these profiles vary within and between populations and whether such variation has any effect on mate choice has not been studied. Cuticular hydrocarbons act as mating pheromones in several insect species, and even slight changes in their composition have been shown to selectively and significantly influence mating success in other species of *Drosophila* (Cobb & Jallon, 1990; Markow & Toolson, 1990).

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For the present study we inbred *D. montana* strains originating from different populations to increase the chance of obtaining strains differing in their mating signals and behaviour. We analysed male courtship songs and the cuticular hydrocarbons of both sexes of five strains and conducted a diallel cross between four of these strains to study the mating propensity and the behaviour of the flies in different intra- and interstrain combinations. The purpose of these studies was to examine how variation in courtship signals affects mate choice, the role of male and female in determining the lengths of different courtship traits and female egg laying efficiency, and which behavioural traits show heterosis in strain crosses. We also investigated the genetic basis of variation in different traits of *D. montana* courtship song using a diallel analysis. Here our goal was to study the presence and direction of dominance in different song traits, only some of which are important in female mate choice, and to trace the direction in which song has evolved. Our prediction was that if female choice drives song evolution the direction of dominance for the traits used in female choice and the direction of female preference should be the same.

Materials and methods

Flies

Strains were inbred for 20 generations by brother–sister matings. Three of the strains originate from Finland: strains 1251 and O7 from Oulanka (66°22′N, 29°21′E; maintained in the laboratory since 1981 and 1985, respectively) and strain K12 from Kemi (65°40′N, 23°35′E 1985). Strain 1263 originates from Kawasaki, Japan (34°80′N, 139°42′E 1969) and strain 1550 from Yukon, Alaska, USA (61°30′N, 159°20′W 1970). This protocol does not allow us to draw conclusions about variation in natural populations as the strains come from different geographical areas and as inbred lines represent natural populations only approximately, especially if there are only a few lines and if the analysed traits exhibit some form of directional dominance (Gebhardt, 1991).

Flies were maintained in culture bottles containing malt medium, in continuous light at 19 °C. This approximates the conditions in the wild when the flies mate at northern latitudes. Larval density was kept constant in culture bottles to avoid variation in the size and condition of emerging flies. Freshly emerged flies were sexed under light CO_2 anaesthesia and maintained separately in food vials. Flies used in experiments were 3 weeks old, and sexually mature.

Hydrocarbon extraction

To extract cuticular hydrocarbons for analysis, a single mature fly was killed by freezing and immersed in 100 μL of hexane. After 5 min, the fly was removed and the solution left overnight in a dust-free environment to allow the hexane to evaporate. After evaporation, the extract was re-dissolved by adding 50 μL of hexane and vortexing for 30 s. A 2-μL sample of the extract was injected into a gas chromatograph (GC) (Varian 3400) fitted with a 15-m, 0.32-mm bore, 1-μm film, DB-1 capillary column (J & W Scientific). Separation of extracted components was optimized by using a column temperature profile in which the analysis began at a temperature of 75 °C rising at 15 °C min^{-1} to 225 °C, and then at 2 °C min^{-1} to 250 °C and finally at 15 °C min^{-1} to 300 °C where the temperature was held for 10 min. Ultra high-purity helium at 5 mL min^{-1} was used as a carrier gas and components were detected using a flame ionization detector. A total of 133 females and 154 males from the five strains were analysed.

Twenty-eight peaks were identified in hydrocarbon analysis. The peaks were standardized to the largest peak as the log of each peak divided by the area of a control peak (Aitchison, 1986). All statistical analyses were carried out using these log contrasts. Principal component analysis was conducted on each sex separately.

Song recording and analysis

Male courtship songs were recorded in a specially designed chamber when a single male courted a single female. The chamber was made of a Petri dish (diameter 5 cm, height 0.7 cm) with a nylon net roof. The floor of the chamber was covered with a moistened filter paper. Males courted females whilst upside down on the roof of the chamber, and the songs were recorded with a Sony TC-FX33 cassette recorder and a JVC-condenser microphone.

Male courtship songs were analysed with the SIGNAL Sound Analysis System (©Engineering Design). In *D. montana*, the males usually produce 3–4 pulse trains of song during the courtship, song characters remaining quite constant once the male begins to sing (Hoikkala & Isoherranen, 1997). Consequently, we analysed for each male three pulse trains of the song, measuring the lengths of the pulse trains (PTL) and counting the number of pulses per train (PN) from oscillograms (see Fig. 1). We also counted the number of cycles (CN) in the third 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 frequencies (FRE) of the pulse trains were measured from Fourier spectra. Distribution of song traits was normal or close to normal and statistical analyses were conducted using nontransformed data. Song traits were also examined using principal component analysis as individual song components may not be independent.

Mating propensity and behaviour of the flies

We observed the behaviour of 19–32 fly pairs for each intra- and interstrain combination of inbred strains O7,

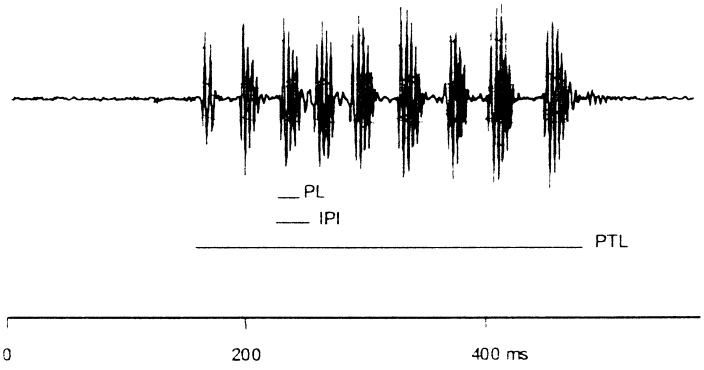

Fig. 1 Oscillogram of one pulse train of the courtship song of a *D. montana* male. PL = pulse length, IPI = interpulse interval and PTL = the length of the pulse train.

1251, 1263 and 1550. Strain K12 was omitted from the analysis because of the low courtship activity of the flies of this strain in preliminary experiments. All flies were 3-week-old mature virgins and had been kept singly in fresh food vials for 24 h prior to the experiments. A male and a female were transferred to a glass vial (height 10 cm, diameter 2.5 cm) containing malt medium and their behaviour was observed for 15 min. For each courting pair we measured length of the latency period (time from the beginning of the experiment to the first courtship act of the male), courtship time (time from the first courtship act of the male to copulation) and copulation duration. These parameters were also measured for F_1 flies emerging from intra- and interstrain crosses, once they were sexually mature (one pair per progeny brood; both male and female from the same progeny).

Flies which did not mate during the observation period were kept in vials for about 2 h. Females mating during this period were included in the progeny production studies, but data for these pairs were not included in the behavioural analysis.

Female egg laying

After the flies had mated, the female was transferred without anaesthesia into a Petri dish (diameter 5 cm, height 0.7 cm) containing a 5-mm layer of malt medium. The Petri dish was covered with a net and a plastic lid. The female was allowed to lay eggs for 3 days, after which she was transferred into a fresh food vial. The number of eggs on the Petri dishes was counted under a microscope.

Distances between strains

To investigate how the pattern of variation in courtships songs and cuticular hydrocarbons relates to the pattern of variation in mating behavioural traits, and female egg laying we conducted distance matrix analysis. First, we calculated Mahalanobis' distances, D^2 (Rao, 1970) between strains for song traits and separately for male and female cuticular hydrocarbon profiles. Using Mantel tests (Manly, 1985), we then compared distance matrices of songs and male and female hydrocarbon profiles with each other and with median distances (Hand, 1981) of the behaviour traits and female egg laying measured between the strains.

Diallel analysis for the male courtship songs

Our diallel data consisted of the songs of the F_1 males from all possible crosses between and within four inbred *D. montana* strains. Males emerging from different Petri dishes (progenies of different males and females) served as repeats (seven repeats). Distribution of song traits was normal, and the statistical analyses were done on nontransformed data. First an ANOVA was conducted to test the variance between males (seven males per genotype) and between genotypes. The variance between genotypes was significant when tested against the within-genotype (i.e. between males) component (P at most <0.05) in all song parameters except PL.

In the diallel analysis we used the means of each male for the studied song traits (three songs from each male). To allow comparison of different parameters, all values (Z) were transformed using $[(Z - \bar{X})/\mathrm{SD}]$ where $\bar{X}$ and SD are the mean and standard deviation, of the same song trait in all males. The diallel data were analysed following Hayman (1954a,b) and Mather & Jinks (1982) using a program written by Jaakko Lumme, Ari-Pekka Kvist and Jouni Aspi.

Results

Hydrocarbons

We conducted a multivariate analysis of variance for cuticular hydrocarbon composition of the flies (MANOVA) using the log-contrast areas for the 28 peaks. This revealed significant differences among strains in cuticular hydrocarbons ($F_{108,998} = 38.74$, $P < 0.001$), and differences between the sexes independent of strain differences ($F_{27,251} = 33.53$, $P < 0.001$). There was also

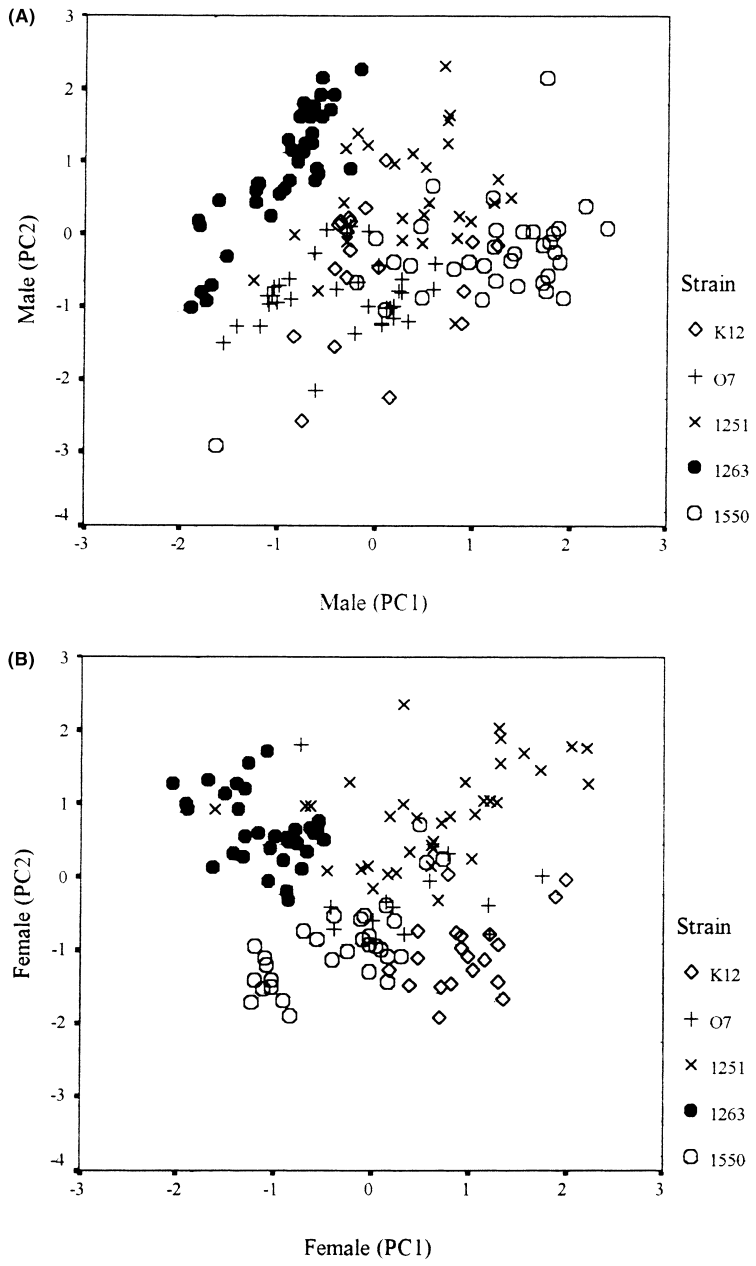

Fig. 2 First and second principal components from analysis of log contrasts of the size of peaks identified by gas chromatography in the cuticular hydrocarbons of five inbred *D. montana* strains: (A) males; (B) females.

significant interaction between strain and sex ($F_{108,998}$ = 7.63, $P < 0.001$), indicating that the difference in cuticular hydrocarbon composition between the sexes varied among strains.

To examine the pattern of divergence between strains in hydrocarbon composition in more detail, we conducted principal component analyses for both sexes. Principal component 1 (PC1) explained 27% of the between-individual variation in males and 30% in females, while PC2 explained 13% and 14%, respectively. Figure 2(A) shows that in males PC1 separates the strains into groups. Strains from Japan (1263) and USA (1550) are most different from each other, while the Finnish strains (K12, 1251 and O7) are grouped together. In females PC1 and PC2 together separate the strains (Fig. 2B). One cannot, however, draw conclusions on differences between populations on the basis of these data.

Because previous work (Bartelt *et al.*, 1986) failed to find any difference in cuticular hydrocarbons between the sexes, we carried out separate MANOVAS of sex vs. PC1 and PC2 for each population separately. This revealed strong differences between the sexes in populations K12, 1550 and O7; but no significant differences in populations 1263 and 1251. (Population 1263, Wilk's

Table 1 Means and standard deviations of different song traits measured for the songs of *D. montana* strains K12, O7, 1251, 1263 and 1550. Means have been calculated over the songs of 10 males per strain (three songs per male). PN = number of pulses in a train, PTL = pulse train length, IPI = interpulse interval, PL = pulse length, CN = cycle number and FRE = carrier frequency.

Strain	PN	PTL	IPI	PL	CN	FRE
K12	8.93 ± 0.64	312 ± 26.4	32.5 ± 2.45	21.3 ± 3.15	4.33 ± 0.48	222 ± 11.9
O7	11.7 ± 1.03	415 ± 40.5	32.7 ± 2.82	17.8 ± 2.57	3.30 ± 0.47	178 ± 15.1
1251	8.97 ± 0.93	322 ± 35.9	34.7 ± 2.10	21.3 ± 3.46	5.00 ± 0.74	236 ± 22.0
1263	9.23 ± 1.33	327 ± 48.7	34.6 ± 3.20	22.3 ± 2.90	4.33 ± 0.48	200 ± 19.2
1550	11.8 ± 1.29	463 ± 64.4	36.5 ± 4.49	25.6 ± 3.11	5.47 ± 0.94	232 ± 20.8

Lamda (Λ) = $\Lambda_{2,69} = 0.96$, $P = 0.26$; Population K12, $\Lambda_{2,36} = 0.44$, $P < 0.0001$; Population 1550, $\Lambda_{2,63} = 0.47$, $P < 0.0001$; Population 1251, $\Lambda_{2,61} = 0.95$, $P = 0.19$; Population O7, $\Lambda_{2,43} = 0.65$, $P < 0.0001$.)

Songs

Six song traits were measured from the male courtship songs of the five *D. montana* strains (Table 1). Variation between strains was significant in all measured song traits (ANOVA, $P < 0.001$ in all cases). To examine the pattern of variation in song traits between the studied strains, we conducted a principal component analysis. PC1 explained 45% and PC2 35% of the variation between individuals. Figure 3 shows that PC1 separates the strains into groups. Strains 1550 (USA) and O7 (Finland) differ most from each other, while the rest of the strains (one from Japan and two from Finland) are more or less mixed.

To interpret the contributions of original variables to each principal component we used the criterion suggested by Mardia *et al.* (1982). Variables with correlations above 0.7 times the largest correlation in an eigenvector were considered to contribute significantly. IPI and all pulse characters PL, CN and FRE correlated with PC1 (Pearson correlations 0.698, 0.860, 0.940 and 0.747, respectively, $P < 0.01$ in all cases), and all four contributed significantly to PC1 (cut-off point = 0.658). PC2 correlated with PN, PTL and FRE (Pearson correlations 0.953, 0.969 and −0.387, respectively, $P < 0.01$ in all cases), but only the pulse train characters PN and PTL contributed significantly to PC2 (cut-off point = 0.678).

The mating propensity and the behaviour of the flies

The mean mating propensity of the flies (defined as the proportion of flies mating during the observation period, e.g. Koepfer, 1987) of P generation was 42.3% in intrastrain combinations and 37.5% in interstrain combinations ($P = 0.761$, NS, Mann–Whitney, $U = 21.5$). The respective propensities for the flies of F_1 generation

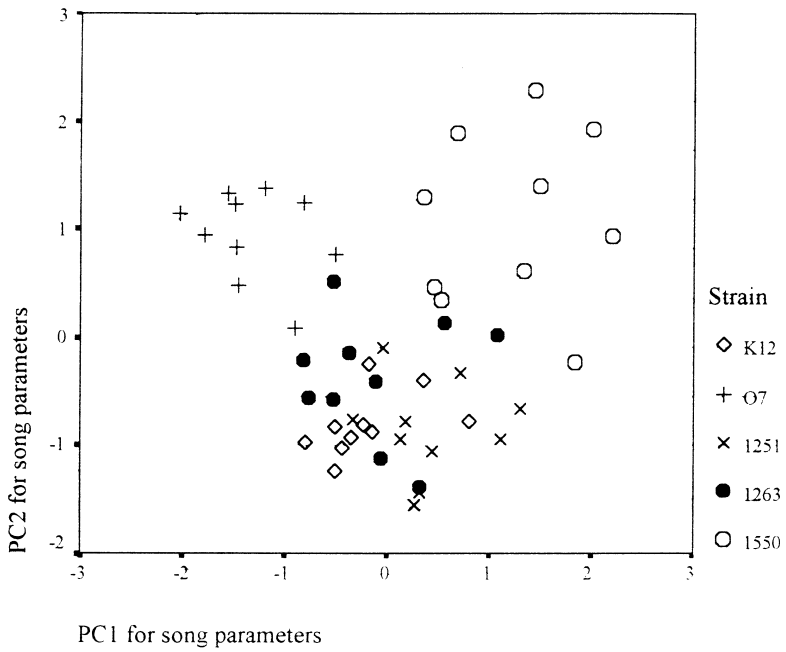

Fig. 3 First and second principal components in the parameters of the courtship song in five inbred *D. montana* strains.

Table 2 Medians (and ranges) of the latencies, courtship durations and copulation durations of the flies which mated during the 15-min observation period, and the number of eggs laid by the females in intra- and interstrain combinations and crosses in two generations (P and F_1) between four inbred *D. montana* strains. *N* refers to the number of courting pairs observed or to the number of females from whom the egg number was calculated.

	P generation				F1 generation			
	Intrastrain combinations		Interstrain combinations		Intrastrain crosses		Interstrain crosses	
	N		*N*		*N*		*N*	
Latency (s)	40	215.5 (17–739)	116	179.5 (4–860)	26	162.0 (31–587)	63	127.0 (21–871)
Courtship duration (s)	40	48.0 (4–661)	116	20.0 (2–464)*	26	96.5 (5–754)	63	20.0 (2–527)†
Copulation duration (s)	40	207.5 (147–351)	116	199.5 (47–443)	26	210.5 (128–435)	63	201.0 (135–369)
Number of eggs	52	30.0 (0–83)	155	25.0 (0–72)	35	27.0 (0–61)	83	30.0 (0–88)

*Courtship duration differed in P generation, Mann–Whitney, $U = 1739$, $P < 0.05$. †Courtship duration differed in F_1 generation, Mann–Whitney, $U = 505$, $P < 0.01$.

obtained from intra- and interstrain crosses were 62.8% and 74.8% ($P = 0.362$, NS, Mann–Whitney, $U = 16.5$).

Medians and ranges of the latency periods, and courtship and copulation durations in successful courtships of the flies in P combinations and F_1 crosses are given in Table 2. Only courtship duration differed significantly between intra- and interstrain combinations in P generation and between intra- and interstrain crosses in F_1 generation (Table 2). In both generations the interstrain combinations or crosses showed shorter courtships than the intrastrain ones.

In combined data of the 16 intra- and interstrain combinations in P generation, the mating propensity of the flies was significantly affected by the female strain (Kruskal–Wallis, $H = 21.17$, d.f. $= 3$, $P < 0.05$). The mating propensity of both males and females of strains O7 (29.8 and 28.3%) and 1251 (32.5 and 26.5%) was low compared to that of the sexes of strains 1263 (48.3 and 47%) and 1550 (44.3 and 53%), respectively. The role of the two sexes in determining the length of the latency period, and courtship and copulation duration in this generation was analysed with Kruskal–Wallis nonparametric analysis of variance. Female strain significantly affected both latency and copulation duration ($H = 7.85$, d.f. $= 3$, $P < 0.05$, and $H = 48.48$, d.f. $= 3$, $P < 0.001$, respectively). Females of strain 1550 were most attractive and females of strain 1251 least attractive, i.e. contributed to shortest and longest male latencies, respectively (1550: median 159 s; 1251: 287 s). Median copulation durations of the females of strains 1550, 1251 and O7 were short (185 s, 191 s and 197 s, respectively) compared to those of females of strain 1263 (241 s). Male strain had no effect on any of the behavioural traits measured.

Female egg laying

Female egg laying was studied in intra- and interstrain combinations in P generation, and in intra- and interstrain crosses in F_1 generation. In P generation, the females of the four inbred *D. montana* strains differed significantly from each other in their egg laying capacity (Kruskal–Wallis, $H = 34.65$, d.f. $= 3$, $P < 0.001$). Also the strain of the male had a significant effect on the number of eggs laid by his partner (Kruskal–Wallis, $H = 8.76$, d.f. $= 3$, $P < 0.05$), even though variation between males was much smaller than between females. Female egg laying did not differ significantly between different combinations or crosses (Table 2).

Spearman rank correlation coefficients were calculated between behavioural traits (latency, mating speed and copulation duration) and female egg laying in P generation. Females of different strains were analysed separately, because female origin had been found to affect most traits. Female egg laying was not correlated with any of the behavioural traits measured.

Distances between strains

Distance matrices for the pattern of variation between strains in courtships songs and cuticular hydrocarbons showed some interesting correlations with matrices for variation in fly behavioural traits or female egg laying in crosses between the four strains of *D. montana* (Mantel tests, Table 3). The highest correlations were between the pattern of variation in male cuticular hydrocarbons and male courtship duration ($0.01 < P < 0.05$), between female cuticular hydrocarbons and female latency ($0.01 < P < 0.05$) and between male songs and female courtship duration ($P \sim 0.05$). The increased chance of a type 1 error due to the large number of tests conducted means that there are no correlations, which we can be confident are significant. The results suggest, however, that male and female hydrocarbons and male songs may affect the attractiveness of the flies as a mating partner.

Diallel analysis for male songs

The means of different song traits of the males obtained from intra- and interspecific crosses are given in Table 4 and the results of the diallel analysis in Table 5. The ANOVA in the diallel analysis revealed significant

Table 3 The correlations (g) and their significance (P) in Mantel tests comparing the distance matrices of four *D. montana* strains (O7, 1251, 1263 and 1550). Distances used are Mahalanobis' distances for song traits and for male and female cuticular hydrocarbon profiles, and median distances for the mating behaviour traits and female egg laying measured between the strains.

	Songs		Male pheromones		Female pheromones	
	g	P	g	P	g	P
Analysis by the male strain						
Male pheromones	0.48	0.3156				
Latency	−1.08	0.1401	−0.87	0.1922	−0.63	0.2643
Courtship duration	0.76	0.2236	1.74	0.0409	1.47	0.0708
Copulation duration	1.16	0.1230	−0.87	0.1922	−1.32	0.0935
Number-of-eggs	−1.17	0.1210	−0.83	0.2033	−0.03	0.4880
Analysis by the female strain						
Female pheromones	−1.06	0.1446	1.54	0.0618		
Latency	1.33	0.0918	−1.11	0.1335	−1.68	0.0465
Courtship duration	−1.62	0.0526	−0.23	0.4090	1.25	0.1056
Copulation duration	−0.62	0.2676	0.34	0.3669	0.69	0.2451
Number-of-eggs	0.38	0.3520	−0.85	0.1977	−1.40	0.0808

Table 4 Means of the song traits of male progenies from intrastrain (bold) and interstrain crosses between inbred *D. montana* strains. N = 7 males in each case. PN = number of pulses in a train, PTL = pulse train length, IPI = interpulse interval, PL = pulse length, CN = cycle number and FRE = carrier frequency.

Female strain	Male strain	Song trait					
		PTL	PN	IPI	CN	FRE	PL
1550	1550	**378**	**11.3**	**29.7**	**5.3**	**255**	**21.3**
1550	O7	336	10.9	28.7	4.4	262	17.1
1550	1263	315	9.3	30.0	5.1	249	24.1
1550	1251	368	10.6	32.4	4.7	256	21.1
O7	1550	317	9.5	32.3	4.2	249	17.3
O7	O7	**358**	**10.6**	**31.4**	**4.0**	**221**	**19.1**
O7	1263	296	8.6	33.3	5.0	256	20.3
O7	1251	315	8.9	34.0	5.0	242	19.7
1263	1550	345	10.3	29.9	4.4	261	17.6
1263	O7	336	9.6	32.7	5.7	261	21.4
1263	1263	**284**	**8.3**	**32.3**	**4.4**	**236**	**18.4**
1263	1251	292	8.3	34.3	5.3	247	20.7
1251	1550	350	10.1	31.4	5.1	262	20.6
1251	O7	354	10.1	33.3	4.3	254	17.0
1251	1263	314	8.9	31.7	5.3	270	19.6
1251	1251	**294**	**8.0**	**35.7**	**5.7**	**262**	**21.4**

variation between genotypes in all song traits, except PL (pulse length), which was not included in further analyses. There was significant additive variance between arrays (a) (i.e. progenies having one common parent) and between parental lines (a_p) in PN (pulse number), IPI (interpulse interval), CN (cycle number) and FRE (carrier frequency) (P at least <0.05). Dominance (b) was found in CN and FRE ($P < 0.05$ in both cases). In CN it was mainly caused by unequally distributed dominant alleles between the strains (b_2, $P < 0.05$), while in FRE the dominance was directional (b_1, $P < 0.01$). No significant residual dominance effects (b_3) nor maternal effects (c) were found. Reciprocal crosses differed from each other in PN (d, $P < 0.05$). However, when tested against specific interactions, d remained nonsignificant, indicating that there are no consistent differences between the reciprocal crosses.

Genetic parameters of the diallel analysis can be estimated further only if the simple additive-dominance model adequately explains variation in the studied data set (see Mather & Jinks, 1982). This can be tested by studying the relationship between the variance (V_r) and parent–offspring covariance (W_r) of the members of the same array. If dominance is present, and if the model is an accurate description of the system, $W_r - V_r$ should be homogenous across strains and a regression of W_r on V_r should produce a straight line with a slope of unity. In our data set $W_r - V_r$ was homogenous across lines in all studied song traits (PTL: $F = 1.439$, PN: $F = 1.641$, IPI: $F = 1.344$, CN: $F = 1.872$ and FRE: $F = 1.473$; $\text{d.f.}_1 = 6$ and $\text{d.f.}_2 = 18$ and P nonsignificant for all traits). The regression slopes of W_r on V_r of PTL (0.283), PN (1.029), CN (0.346) and FRE (0.664) did not differ significantly from unity (PTL: $t = 1.146$, PN: $t = 2.455$, CN: $t = 2.514$ and FRE: $t = 4.00$; d.f. = 2 and P nonsignificant in all cases), but they did not differ from zero, either (PTL: $t = 0.323$, PN: $t = 2.517$, CN: $t = 0.871$ and FRE: $t = 2.641$; d.f. = 2 and P nonsignificant in all cases). On

Table 5 Analysis of variance on the diallel tables of sound parameters and the diallel components. The mean squares of the items are tests for the following effects: a = additive effects between arrays, a_p = additive effects between parental lines, b = dominance effects, b_1 = effects of directional dominance, b_2 = effects of the distribution of dominant alleles between strains, b_3 = residual dominance effects, c = maternal effects, d = differences in reciprocal crosses. PN = number of pulses in a train, PTL = pulse train length, IPI = interpulse interval, PL = pulse length, CN = cycle number and FRE = carrier frequency.

		PTL		PN		IPI		CN		FRE	
	d.f.	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Total	111										
Between blocks	6	0.67	0.74	0.60	0.82	1.15	1.45	0.53	0.61	0.55	0.65
Between genotypes	15	1.67	1.84*	2.77	3.78***	2.19	2.77*	1.96	2.26**	2.10	2.49**
Within genotypes (e)	90	0.91		0.73		0.79		0.87		0.85	
a	3	1.91	2.10	7.64	10.43***	8.57	10.82***	3.20	3.67*	2.76	3.26*
a_p	3	1.76	1.94	5.02	6.86***	2.90	3.67*	2.79	3.21*	4.14	4.89**
b	6	1.84	2.03	1.25	1.71	0.45	0.57	2.12	2.43*	2.32	2.74*
b_1	1	0.12	0.14	0	0	0.54	0.68	2.28	2.62	7.15	8.44**
b_2	3	2.24	2.46	1.60	2.18	0.61	0.77	2.42	2.78*	1.81	2.13
b_3	2	2.11	2.32	1.36	1.86	0.16	0.20	1.57	1.81	0.68	0.80
c	3	0.99	1.09	1.60	2.19	1.12	1.42	1.04	1.20	1.86	2.19
d	3	1.77	1.95	2.09	2.85*	0.37	0.47	1.35	1.55	1.26	1.49

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

the other hand, in IPI (regression slope 0.842) the mean values of different strains were on a linear regression line and the slope of this trait differed significantly both from unity ($t = 11.99$, d.f. $= 2$, $P < 0.01$) and from zero ($t = 9.90$, d.f. $= 2$, $P < 0.05$). The fact that our data did not completely fulfil the assumptions of the additive-dominance model does not undermine the findings of dominance in CN and FRE, but it suggests that the song traits may be affected in addition to additive and dominance variation also by epistatic interactions between nonallelic genes, by multiple allelism and/or by correlated allele distributions.

Discussion

Male song traits and cuticular hydrocarbons of both sexes varied between inbred *D. montana* strains, giving a good basis for studies on fly mate choice. The strains also differed in flies' mating propensity, some behavioural traits and female egg laying efficiency. The flies mated as actively in inter- as in intrastrain combinations, showing that variation between strains in studied traits did not cause sexual selection or sexual isolation between strains. Only courtship duration and carrier frequency of the male song showed heterosis in strain crosses.

According to Bartelt *et al*. (1986), *D. montana* males and females have virtually identical hydrocarbon profiles. However, we found considerable variation both between the strains and between the sexes in cuticular hydrocarbon composition. A likely explanation for this discrepancy is that the strains used differ between the two studies. We found considerable variation among our strains, and three out of the five strains showed dimorphism in cuticular composition. Variation found in our populations could also be partly due to inbreeding and to the maintenance of the flies in the laboratory. Toolson & Kuper-Simbron (1989) have shown that long-term maintenance of the fly strains (*D. pseudoobscura*) under laboratory conditions can lead to significant changes in cuticular hydrocarbon composition and cuticular permeability in both male and female flies. In our study, the length of the male latency period was found to be influenced by the female, suggesting that the females of different strains varied in their attractiveness. This could be due to pheromones emitted by the females as the females preened (cleaned themselves with forelegs) actively, which could have released low-volatility cuticular hydrocarbons. There was, however, no correlation between male latency and the divergence of the cuticular composition of females courted by these males.

Averhoff & Richardson (1974) reported that in inbred *D. melanogaster* lines, courtship activity, time to copulation and assortative mating were all directly correlated with the degree of inbreeding. They suggest this to be due to the flies being nonresponsive to their own pheromones and therefore unresponsive to individuals of very similar genotype. This negative assortative mating has not, however, been confirmed by later studies (Powell & Morton, 1979; Veuille & Mazeau, 1988). In our study flies mated as actively with individuals of their own strain as with those of alien strains, even though the hydrocarbon profiles of the strains differed considerably. Females, however, required a shorter courtship from the males of other strains than from the males of their own strain in P generation. Whether this higher level of stimulation from alien males is due to song or pheromonal differences between strains is difficult to assess.

The finding that male strain affects the number of eggs laid by his partner is particularly intriguing. It is possible that there are differences between strains in some aspect of male ejaculates. There is some evidence that ejaculates influence female egg laying (Pitnick, 1991), so the effect of males may be due to differences in the ability of their ejaculates to stimulate females to oviposit. Alternatively, differences between male strains in female egg laying could be due to females exercising 'cryptic female choice' (Eberhard, 1996). If particular combinations of strains, or matings within strains, are liable to produce less fit offspring (due to genetic incompatibilities resulting from inbreeding or outbreeding), females may withold some of their eggs, in the hope of a subsequent mating with another male.

Inbred *Drosophila* strains have repeatedly been shown to suffer decreased fitness and to show heterosis in strain crosses (Ehiobu *et al*., 1989; López-Fanjul & Villaverde, 1989; García *et al*., 1994; see also Lynch & Walsh, 1997, for a survey of inbreeding depression in *Drosophila* laboratory populations). Amongst other traits, inbreeding depression has been observed in competitive ability, egg-to-adult viability, male mating ability and female and male fertility. In our strains, the homozygosity level of the flies should theoretically exceed 90% (e.g. Hedrick, 1985) although homozygosity is typically slightly lower than predicted (Franklin, 1977). We found evidence for heterosis only in courtship duration and the carrier frequency of male song. Our study does not, however, give information on whether heterosis in courtship duration is caused by bringing together dominant favourable genes of both parents in the hybrid, or by heterozygosity *per se*.

Songs of inbred *D. montana* flies exhibited considerable variation between strains. PCA results suggested that all measured song characters were important in defining the song as strain specific, pulse characters being slightly more important. The diallel analysis on male song traits revealed unidirectional dominance in the carrier frequency of the song, alleles increasing the frequency being dominant over those decreasing the frequency. On the basis of the direction of dominance, the song of *D. montana* is likely to have evolved towards higher song frequency. The females of this species are known to prefer males producing songs consisting of short and dense (high-frequency) sound pulses (Aspi & Hoikkala, 1995; Ritchie *et al*., 1998), and females have also been found to gain indirect benefit from their choice (Hoikkala *et al*., 1998). In *D. montana* the direction of female preference for male song is the same as the direction of song evolution, i.e. sexual selection exercised by the females could have been a driving force in the evolution of at least some male song traits.

Signals important in sexual selection within the species may be different from those important in species-recognition. Consequently, mate choice exercised by the females on conspecific males may not explain the evolution of all male signals. As noted by Civetta & Singh (1998), directional sexual selection at the time of species formation may have triggered the rapid divergence observed for sexual traits, but once speciation is completed, the selective pressure may have relaxed. In the present study, the traits by which the song of *D. montana* males differs most from those of the other species of the *D. virilis* group (Hoikkala *et al*., 1982) did not show any sign of directional dominance in diallel analysis. This does not exclude the possibility that directional selection for species-specific songs has triggered the divergence of male song traits (other than carrier frequency) at the time of species formation, but it shows that this kind of evolution has not been a feature of the recent history of *D. montana*.

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