Chromosomal effects on male and female components of sperm precedence in *Drosophila*

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Summary

Recent experiments with *Drosophila* have demonstrated that the success of sperm in multiply mated females depends on the genotype of both the male and the female. To further characterize the distinction between male and female roles in sperm success, we scored variation in both sexes in sperm competitive ability among a set of chromosome replacement lines that allow identification of effects to each chromosome. We detected significant male and female effects on sperm precedence, defined as the ability of a male's ejaculate to displace resident sperm (*P2*) or avoid being displaced by subsequent matings (*P1*). Tests of effects of first, second and third chromosome substitutions revealed significant differences among third chromosomes in male sperm precedence (both *P1* and *P2*) and a first × second chromosome interaction in female's effect on sperm precedence (only *P1*). We found no significant correlation between male and female effects on sperm precedence, suggesting that the variation found in both *P1* and *P2* has a different genetic cause in the two sexes.

1. Introduction

Despite their obvious role in the determination of mating success, male traits that are subjected to sexual selection generally exhibit an abundance of genetic variation. High heritability has been associated with different components of male courtship in a wide variety of animal groups (Cade, 1984), and the coefficient of additive genetic variance was found to be significantly higher for sexually selected traits than non-sexual traits (Pomiankowski & Møller, 1995). Sperm competition can be considered as one particular example of postmating male–male competition between mates. In *Drosophila*, there is extensive evidence for variation among males in sperm competitive ability. Remating and multiple paternity is common in both natural populations and laboratory strains (Gromko *et al.*, 1984*a*; Marks *et al.*, 1988; Harshman & Clark, 1998), and sperm competition has been demonstrated by double mating single females to wild-type and morphologically marked strains and counting the proportion of progeny obtained from each male (Lefevre & Jonsson, 1962; Parker, 1970;

Prout & Bundgaard, 1977; Gromko *et al.*, 1984*b*; Newport & Gromko, 1984; Clark *et al.*, 1995).

Extensive genetic variation has been found for the fraction of progeny sired by a second male (*P2*) (Gromko *et al.*, 1984*a*). Clark *et al.* (1995) tested males from 152 lines of *D. melanogaster* that were homozygous for second or third nature-extracted chromosomes against females and males from a *cn bw* stock. Extensive genetic variation was found among the different lines in their ability to displace sperm or resist displacement by the *cn bw* control males, and alleles from four accessory gland protein genes showed significant association with the sperm's ability to resist displacement (i.e. defence), but not with its ability to displace resident sperm (i.e. offence). Hughes (1997) partitioned the genetic variance for sperm precedence among third chromosome homozygous lines and, based on estimates of inbreeding decline, suggested that a few genes of large homozygous effect influence sperm precedence.

Eberhard (1996) has shown examples of variation associated with the females' decision to 'choose' a mate, not only before but also during and after copulation. However, relatively little is known about the amount of genetic variability underlying female choice and female–male interactions that may control

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sperm usage and paternity. Clark & Begun (1998) have recently found extensive genetic variation among females from different chromosome extracted lines for their ability to differentially use two alternative sperm sources. Some lines behaved as 'mixers' whereas others strongly favoured one of the two males' sperm. These results suggest that females also have a role in determining which sperm is used during fertilization, and a significant male–female interaction in sperm usage has been detected in both offence and defence components of sperm displacement (Clark *et al.*, 1999).

The extensive variation observed for both male and female components of sperm precedence raises the question of how such extensive polymorphism is maintained in populations. By examining the possible outcomes of a model with three male genotypes mated to any female type, Prout & Bundgaard (1977) found that polymorphism in sperm precedence could be maintained by either heterosis or some form of non-transitive pattern of sperm displacement parameters among male genotypes. A more recent model extends the previous finding to situations in which allelic variation in sperm displacement has pleiotropic effects on fecundity and on mating success (Prout & Clark, 1996), and it is also possible that the extensive genetic variation observed in both males and females might be maintained by specific male–female allelic interactions (Clark *et al.*, 1999). These studies have assumed a genetic basis for sperm precedence, based on the extensive variation detected among isogenic lines, but we are still lacking studies that attempt to map the genetic factors underlying this phenotype.

In this study, we used a set of 20 chromosome-substitution lines to quantify the effect of chromosome replacement in offence and defence components of sperm displacement, and we assessed whether sex-specific effects on sperm displacement are co-localized in the genome.

2. Materials and methods

(i) *Drosophila cultures*

Chromosome substitution lines between three iso-female lines from France (FrV3-1), California (Hg), and Zimbabwe (Z30) were kindly provided by Dr Chung-I Wu and are described in Wu *et al.* (1995) and Hollocher *et al.* (1997) (Table 1). We note that these lines were not initially co-isogenic, but were highly inbred as described in Hollocher *et al.* (1997). The females and control males used to analyse the male aspects of sperm precedence were a laboratory stock of *cn bw* (bearing the second chromosome recessive alleles of *cinnabar* and *brown*, producing white eyes), obtained from the Bloomington Stock Center. For the analysis of the female component of sperm precedence,

Table 1. *Chromosome substitution lines used in this study*

Stocks	Chromosome substitutions		
	X	II	III
1, 4	M	M	M
8, 14	M	M	Z
9, 15	M	Z	M
10, 16	M	Z	Z
11, 17	Z	M	M
12, 18	Z	M	Z
13, 19	Z	Z	M
6	Z	Z	Z

Stocks	Third-chromosome arm substitutions	
	Left arm	Right arm
1, 6	+	+
2, 7	+	R
3, 20	R	+
5	R	R

M refers to either France or California (for the left and right line index numbers, respectively), and Z is used for Zimbabwe origin. For the third chromosome arm substitution lines, + means either France or Zimbabwe and R is used for *ru cu ca* balancer stock origin. The assigned stock numbers are arbitrary.

males were taken from a bw^D laboratory stock (a dominant allele of the *brown* locus, with a brown-eye phenotype, kindly supplied by Dr Mel Green, UC Davis), and the control stock was a third chromosome isogenic line B3-09, kindly provided by Dr Brian Charlesworth, University of Edinburgh. B3-09 is also homozygous for the fourth chromosome recessive *sparkling*^{poliert} which gives red glassy eyes.

(ii) *Variation in male sperm precedence*

Throughout this experiment, females were from the *cn bw* strain, and all 20 chromosome replacement lines were tested against *cn bw* males. For each test the female was mated to *cn bw* and the tested line of male in both orders. If the tested male is the second to mate, the test measures the offence component, or the ability of the tested male's sperm to out-compete resident *cn bw* sperm. If the *cn bw* male had mated second, the test measures the ability of the tested male's sperm to defend against being displaced by the *cn bw* sperm. In both experiments, virgin 4- to 5-day old females were mated first to same-aged, virgin *cn bw* (or chromosome replacement lines) males *en masse* for two hours. Females were then aspirated into individual vials, where they were allowed to oviposit for 2 days. These vials were designated as 'vial 1'. Then two or three males of the same chromosome replacement (or *cn bw*) line were placed in each vial for the second mating

and left overnight. Second males were then removed and females were transferred by aspiration to vial 2. After 4 days females were transferred again without anaesthesia to vial 3, and a week later females were discarded. All three vials were scored for eye colour phenotype (wild vs *cn bw*) on the 17th day after oviposition began. Only sets of three vials that yielded the two possible phenotypes were scored, and the fraction of all progeny in vials 2 and 3 that were sired by the second male was designated as the statistic *P2* (Boorman & Parker, 1976). Fecundity was scored as the total count of progeny produced by each female summed over her oviposition vials. Crosses that produced fewer than 20 flies (fecundity < 20) were eliminated from the analysis.

(iii) *Variation in female components of sperm precedence*

The same 20 lines that were used to compare male components of sperm precedence were also used in tests of effects of female genotypes. Each female line was crossed to B3-09 and bw^D males in both orders, following the same protocol of virgin collecting, ageing and timing for the two matings described in the previous section.

(iv) *Viability assays*

A positive and significant correlation between larval viability and sperm displacement ability has been found previously (Gilchrist & Partridge, 1997), suggesting that any attempt to discern the genetic basis of sperm precedence should take into consideration possible pre-adult viability effects. Therefore, we started two independent sets of crosses to quantify the egg-to-adult viability of the genotypes scored in the sperm precedence experiments. Viability for the male side of the experiments was tested by crossing *cn bw* 4- to 5-day old virgin females to same-aged males from each of the chromosome substitution lines in a 1:1 ratio. Two or three hybrid *cn bw*$/+_i$ males obtained from each of these crosses (where $+_i$ represents the 20 different chromosome substitution lines tested) were then set up in individual vials containing one *cn bw* virgin female. All vials were scored for eye colour phenotype (wild vs *cn bw*) on the 17th day after oviposition began and viability was measured as the ratio of *cn bw* to wild-type flies obtained from each cross.

   The viability of the genotypes scored in the female side of the sperm precedence experiments was assessed by first crossing 4- to 5-day old virgin females from the bw^D stock to males of the B3-09 line. B3-09/bw^D hybrid males were then crossed to virgin females from each of the chromosome substitution lines as previously described. All vials were scored for eye colour

(wild vs. bw^D) on the 17th day after oviposition began and viability was measured as the ratio of bw^D to wild-type flies obtained from each cross.

(v) *Multiple matings*

There is a chance that by allowing the second matings to occur overnight, females may mate with a second and possibly third (or more) males. This will introduce a bias into the scores of male and female effects on sperm precedence if males differ in their ability to restrict females from engaging in subsequent matings or if females make differential usage of stored sperm. Following the same crossing schemes described in Sections 2(ii) and 2(iii), a new set of 891 females were observed every 10 min for multiple matings occurring overnight (from 4 p.m. to 9 a.m.).

(vi) *Statistical analysis*

Three-way analysis of variance was used to test the effect of different chromosome replacement on sperm precedence. The model is

$$Y_{ijkl} = \mu + a_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl}$$

Y_{ijkl} represents the *P1* or *P2* value for replicate l having first, second and third chromosomes indexed i, j and k, where the indices represent the origin of the chromosome (i.e. France, California, Zimbabwe). α_i, β_j and γ_k are the fixed treatment effects for chromosomes 1, 2 and 3 respectively. The tests were done separately for the offence and defence components of both male and female effects on sperm precedence. For hypothesis testing of sperm competitive effects, both *P2* and angular transformed *P2* values were used to quantify mean squares between chromosomes separately for male and female effect experiments, but they were generally consistent so only the transformed statistics are reported. Spearman rank correlations among *P1*, *P2* and fecundity were also tested. All statistical tests were done using the SAS STAT package version 6.03.

3. Results

(i) *Viability corrections*

The proportions of wild-type and mutant eye-colour progeny obtained in the viability test crosses are shown in Table 2. The overall test of heterogeneity across lines in segregation ratios (Table 2) was found to be non-significant both relative to *cn bw* for the male component tests ($F_{19, 238} = 0.73$, $P = 0.784$) and relative to bw^D for the female component tests ($F_{19, 175} = 0.89$, $P = 0.594$). Given that the estimates of female and male effects on sperm precedence could be affected by the egg-to-adult viability of the eye-mutant

Table 2. *Mean proportion of wild-type progeny (p) segregating from crosses using a set up to test for viability effects on the male or female side of sperm precedence experiments*

Substitution line	Male			Female		
	p	SE	n	p	SE	n
F,F,F	0·552	0·017	357	0·492	0·022	573
F,F,Z	0·583	0·022	357	0·494	0·018	368
F,Z,F	0·525	0·042	340	0·502	0·019	499
F,Z,Z	0·535	0·017	253	0·482	0·022	321
Z,F,F	0·509	0·020	229	0·487	0·025	532
Z,F,Z	0·570	0·017	333	0·516	0·035	788
Z,Z,F	0·564	0·014	320	0·472	0·028	404
Z,Z,Z	0·570	0·025	292	0·646	0·028	40
H,H,H	0·593	0·034	261	0·534	0·024	581
H,H,Z	0·494	0·020	352	0·482	0·042	395
H,Z,H	0·537	0·027	356	0·473	0·022	549
H,Z,Z	0·589	0·034	256	0·522	0·014	343
Z,H,H	0·565	0·023	217	0·499	0·023	344
Z,H,Z	0·588	0·030	269	0·491	0·017	238
Z,Z,H	0·553	0·034	308	0·480	0·024	699
R,R,R	0·522	0·042	302	0·513	0·034	554
F,F,F.R	0·544	0·048	303	0·482	0·031	287
F,F,R.F	0·510	0·047	306	0·497	0·024	385
Z,Z,Z.R	0·557	0·034	235	0·490	0·019	311
Z,Z,R.Z	0·560	0·017	326	0·491	0·022	589

The chromosomal substitution lines are listed by their chromosomal constitution, where commas and full stops are used to separate single chromosome and chromosome arm origins respectively. SE and *n* stand for standard error and sample size, respectively.

strain used as a tester, we corrected all our estimates of sperm precedence. The correction was done for each line by dividing the actual counts from each vial by $2k$ (where k is the segregation fraction of respective wild-type or mutant flies obtained from the viability tests) (Clark *et al.*, 1999).

(ii) *Multiple matings*

Each female was observed to mate once or twice overnight after the first mating, but no triple or higher number of matings occurred. Only 19 of the original 20 chromosome extracted lines were tested since line 5 (Table 1) had been lost.

Among the 260 *cn bw* females that mated first to males from the chromosome extracted lines, only one female engaged in two consecutive matings with *cn bw* males. Five of 263 *cn bw* females that mated first to *cn bw* males mated twice overnight with males from the chromosome extracted lines.

A total of 368 females from the chromosome extracted lines were observed for multiple matings occurring overnight. One of 217 females that were first mated to B3-09 males mated twice to bw^D males, and one of 151 flies engaged in two matings with B3-09
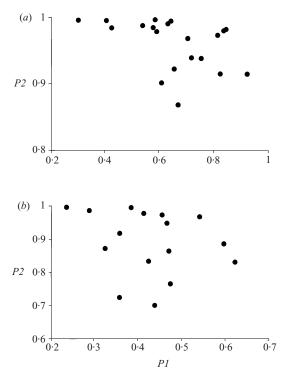

Fig. 1. Offence ($P2$) versus defence ($P1$) mean scores obtained for different chromosome substitution lines. (*a*) Results for male effects on sperm precedence. (*b*) Results for female effects on sperm precedence.

males after having had a first copulation to bw^D males. These rates of multiple mating were low enough that they can be ignored in subsequent analysis.

(iii) *Male effects on sperm precedence*

Fig. 1*a* shows the variability across the 20 lines in male effects on sperm precedence statistics. The offence component of sperm displacement ($P2$) showed significant heterogeneity among lines ($F_{14,172} = 1·80$; $P = 0·04$), while the defence component ($P1$) was highly significant ($F_{14,102} = 2·29$; $P = 0·009$). The assays for male effects on sperm precedence were well replicated with a total of 68 840 offspring scored from 1133 females (in 3399 vials).

There were significant fecundity differences among chromosome substitution lines in the defence test of sperm displacement ($F_{14,102} = 2·21$; $P = 0·01$) with a significant effect caused by the replacement of the first chromosome ($F_{2,102} = 5·46$; $P = 0·006$) (Table 3). However, the correlation between $P1$ and fecundity was not significant ($r = -0·087$; $P = 0·349$), suggesting that the differences in fecundity are not the result of an association between efficiency of first male's sperm usage and resistance to displacement. Only a marginally significant fecundity effect among lines in the offence test was found ($F_{14,178} = 1·74$; $P = 0·051$) due to a third chromosome effect (Table 3). A significant and positive correlation between $P2$ and

Table 3. *Chromosome substitution effects on fecundity of both mutant and wild-type females in tests of male and female components of sperm competition*

(a) *Male component*

Chromosome effect	Fec$^+$				Fec$^{cn\,bw}$			
	df	M.S.	F	P	df	M.S.	F	P
1st	2	218	0·33	0·723	2	3967	5·46**	0·006
2nd	2	141	0·21	0·811	2	454	0·63	0·537
3rd	2	2473	3·68*	0·027	2	1079	1·48	0·232
1st × 2nd	2	1278	1·90	0·153	2	1939	2·67	0·074
1st × 3rd	2	785	1·17	0·313	2	1612	2·22	0·114
2nd × 3rd	2	1484	2·21	0·113	2	1364	1·88	0·158
1st × 2nd × 3rd	2	129	0·19	0·826	2	334	0·46	0·633

(b) *Female component*

Chromosome effect	Fec$^+$				Fec^{bwD}			
	df	M.S.	F	P	df	M.S.	F	P
1st	2	320	2·21	0·113	2	2429	10·54***	0·0001
2nd	2	119	0·82	0·441	2	605	2·62	0·075
3rd	2	614	4·24*	0·016	2	431	1·87	0·157
1st × 2nd	1	268	1·86	0·175	2	122	0·53	0·590
1st × 3rd	1	114	0·79	0·376	2	410	1·78	0·172
2nd × 3rd	1	724	5·00*	0·027	2	64	0·28	0·756
1st × 2nd × 3rd	0				0			

All analyses are based on viability-corrected data. Details of the ANOVA model are in the text.
* $0·01 < P < 0·05$; ** $0·001 < P < 0·01$; *** $P < 0·001$.

Table 4. *Individual chromosome effects on male and female components of sperm precedence. All analyses are based on viability corrected data*

(a) *Male component*

Chromosome effect	P2				P1			
	df	M.S.	F	P	df	M.S.	F	P
1st	2	0·090	2·40	0·094	2	0·017	0·33	0·722
2nd	2	0·004	0·11	0·900	2	0·068	1·30	0·276
3rd	2	0·151	4·02*	0·020	2	0·341	6·50**	0·002
1st × 2nd	2	0·045	1·20	0·304	2	0·138	2·63	0·077
1st × 3rd	2	0·105	2·79	0·064	2	0·106	2·03	0·137
2nd × 3rd	2	0·007	0·18	0·835	2	0·057	1·09	0·339
1st × 2nd × 3rd	2	0·043	1·16	0·318	2	0·000	0·00	0·999

(b) *Female component*

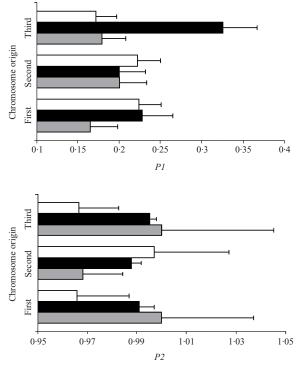| Chromosome effect	P2				P1			
	df	M.S.	F	P	df	M.S.	F	P
1st	2	0·317	2·04	0·134	2	0·320	1·74	0·179
2nd	2	0·381	2·45	0·089	2	0·341	1·85	0·160
3rd	2	0·138	0·89	0·413	2	0·121	0·65	0·521
1st × 2nd	1	0·423	2·72	0·101	2	0·599	3·25*	0·041
1st × 3rd	1	0·021	0·14	0·711	2	0·317	1·72	0·182
2nd × 3rd	1	0·315	2·02	0·157	2	0·353	1·92	0·150
1st × 2nd × 3rd	0				0			

* $0·01 < P < 0·05$; ** $0·001 < P < 0·01$.

Fig. 2. Male components of sperm precedence of single chromosome substitutions. The chromosome origin is indicated by a distinct filling pattern for each bar: open bars, California; black bars, France; grey bars, Zimbabwe).
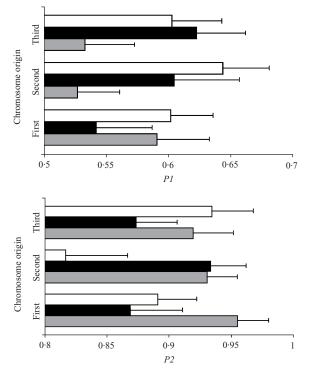

Fig. 3. Female components of sperm precedence of single chromosome substitutions. The chromosome origin is indicated by a distinct filling pattern for each bar: open bars, California; black bars, France; grey bars, Zimbabwe.

fecundity ($r = 0.221$; $P < 0.01$) was detected before the data were corrected for viability effects, but the correlation did not hold after correcting the data ($r = -0.043$; $P = 0.55$).

The analysis of variance (ANOVA) showed significant heterogeneity among third chromosomes on *P2* ($F_{2, 172} = 4.02$; $P = 0.020$) (Table 4, Fig. 2). The defence component of sperm precedence also showed a highly significant third chromosome effect ($F_{2, 102} = 6.46$; $P = 0.002$) (Table 4, Fig. 2). The third chromosome effects on offence and defence were further analysed by using the third chromosome arm substitution lines (Table 1). *P2* showed non-significant differences based on third chromosome arm substitutions ($F_{3, 85} = 0.19$; $P = 0.906$) whereas a significant effect was attributable to a right arm substitution on *P1* ($F_{3, 75} = 4.70$; $P = 0.005$; Tukey-Kramer $\alpha = 0.01$).

(iv) *Female effects on sperm precedence*

Fig. 1*b* shows the variability across the 20 lines in female effects on sperm precedence statistics. The offence component of sperm precedence (*P2*) was significant ($F_{10, 153} = 2.12$; $P = 0.026$), and the defence component (*P1*) showed highly significant differences among lines ($F_{12, 199} = 2.42$; $P = 0.006$). The assays of female effects on sperm precedence were also well replicated with a total of 56 874 offspring produced by 1544 females scored.

There were significant fecundity differences among chromosome substitution lines in both offence and defence tests of sperm displacement ($F_{10, 161} = 2.77$; $P = 0.003$ and $F_{12, 200} = 4.84$; $P = 0.0001$ respectively), with a significant effect caused by a third and a second × third chromosome interaction for the offence experiment and a first chromosome effect for the defence side of the experiment (Table 3). The genetic basis for fecundity effects is dependent on whether females mate first to the wild-type strain (B3-09) or the marker (bw^D). This discrepancy in chromosomal effects depending on mating order is similar to the results obtained from the experiments designed to map the male components of sperm precedence (Table 3). When the second male is from the wild-type strain (either B3-09 or any substitution line) there is a consistent third chromosome substitution effect in fecundity, and a consistent first chromosome effect is observed when the second male is from the marker stock (bw^D or *cn bw*) (Table 3). However, neither of the two estimates of sperm precedence significantly correlated with fecundity ($r = 0.10$; $P = 0.15$ and $r = 0.09$; $P = 0.26$ for *P1* and *P2* respectively).

The results from the analysis of variance showed a marginally significant effect of substitutions of the second chromosome on *P2* ($F_{2, 153} = 2.45$; $P = 0.089$), and a significant first × second chromosome interaction on *P1* ($F_{2, 199} = 3.25$; $P = 0.041$) (Table 4, Fig. 3).

(v) *Male × female interactions*

Although the substitution lines were tested in separate experiments for their male and female effect on sperm displacement, it is possible to determine whether some form of male–female interaction affects sperm precedence. Such a test asks whether the females from lines in which males are strong displacers are promoters or detractors of sperm displacement. Because the tests of female components of sperm precedence essentially test the tendency of females to discriminate between first and second males, we considered the highest, lowest and average of the *P1* and *P2* values when estimating correlations with male effects, but results were similar so only correlations with the averages are reported. The correlation between male *P2* and female sperm discrimination was negative but non-significant ($r = -0.363$; $P = 0.167$) while male *P1* showed a positive and marginally significant correlation with female sperm discrimination ($r = 0.467$; $P = 0.068$). The lack of a significant correlation between males and females in sperm competition effects further supports the idea that different genes are involved in the trait in males and females, but this test is not very powerful because of the limited number of lines assayed.

4. Discussion

Differences among these chromosome substitution lines in male components of sperm precedence are mainly caused by genes on the third chromosome, while a first × second chromosome effect was found for the female's effect on sperm precedence (Table 4). The lack of co-localization of male and female effects on sperm precedence suggests that variation in these two phenomena have a separate genetic basis.

We observed that fewer than 2% of the singly-mated females re-mated with more than one male in the overnight test. Even if all of those cases of multiple mating resulted in complete sperm precedence, the impact on the ANOVA results would be minor due to the rarity of multiple matings. We note, however, that an explicit test for such multiple mating may be important in a design of this sort, as some genotypes will undergo multiple mating in overnight trials (L. Partridge, personal communication).

It has been shown previously that once sperm from different males reaches the females' storage organs, there are competitive differences in their ability to fertilize the females' eggs (see Markow, 1997). Extensive genetic variation has been detected among different male genotypes in the ability of their sperm to displace or avoid being displaced by sperm of another male (Clark *et al.*, 1995; Hughes, 1997). However, apart from comparing different series of chromosome extraction lines, there has been no previous attempt to map these differences. Our study shows a common third chromosome effect in detecting significant differences among offence and defence abilities of sperm. Although a significant third chromosome effect on female fecundity was also detected, there was no significant correlation between either of the two estimates of sperm precedence and fecundity, suggesting that despite of the co-localization of the two phenomena on the same chromosome, they may have a different genetic basis. There is also evidence based on the lack of a significant correlation between *P1* and *P2* scores that, despite a common third chromosome effect in offence and defence sperm precedence, different genes might affect such traits (Clark *et al.*, 1995, 1999).

Although our results show a detectable significant effect of only third chromosome substitutions in male sperm precedence, this does not rule out the possible involvement of other candidate single genes located on other chromosomes. For example, Clark *et al.* (1995) have suggested a potential effect of some molecular variants of accessory gland genes located on the second chromosome in the ability of males to resist displacement.

There are several genes on the third chromosome of *D. melanogaster* that, based on our previous knowledge of their physiological effects, might be good candidates for playing a role in sperm precedence. Particularly interesting are genes expressed in the male accessory gland since they have been shown to affect female postmating behaviour (Wolfner, 1997 and references therein) as well as sperm storage and sperm competition (Kalb *et al.*, 1993; Harshman & Prout, 1994). Our knowledge about the physiological effects of the genes for which we currently have sequences available is still limited. However, two of the *Acp* genes localized on the third chromosome, *Acp62F* and *Acp76A*, have shown sequence homologies that make them good candidates for sperm precedence functions. *Acp62F* has sequence similarity to spider neurotoxin. After mating, most of the protein remains in the female storage organs with very low levels entering the circulatory system. It is then feasible that this protein, through its toxicity effect, could regulate sperm storage and usage (Wolfner, 1997). The other accessory gland protein, *Acp76A*, has sequence similarity with protease inhibitors, which could regulate cleavage of other Acps or lead to semen coagulation (Wolfner, 1997). However, Clark *et al.* (1995) have previously shown no association between sperm precedence and allelic variation in the *Acp76A* region among third chromosome extracted lines.

Molecular evolutionary studies of genes encoding accessory gland proteins also provide evidence for their role in determining reproductive fitness. *Acp26A* and *Acp70A* have both shown a high overall estimate

of nucleotide diversity ($\pi = 0 \cdot 007$ and $0 \cdot 016$, respectively) (Aguadé *et al.*, 1992; Cirera & Aguadé, 1997) compared with the average estimate obtained from a survey of genes sequenced in *D. melanogaster* ($\pi = 0 \cdot 004$) (Moriyama & Powell, 1996). The most thoroughly studied *Acp26A* gene has shown a significant excess of amino acid polymorphism and an elevated proportion of amino acid replacements between species, suggesting that directional selection may have shaped the evolution of these genes (Aguadé *et al.*, 1992; Tsaur & Wu, 1997; Aguadé, 1998). It is possible that such rapid evolution may be a common characteristic of sex-related genes functionally involved in mating, fertilization and/or sex-determination (Civetta & Singh, 1998, 1999).

It is becoming clearer that females play an active role in controlling which sperm will fertilize their eggs, and that there is extensive genetic variation underlying the female effects on sperm precedence (Clark & Begun, 1998). No single chromosome effect was detected for female control over sperm precedence. Only a significant first × second chromosome interaction was found for *P1* (Table 4). Substitutions of the first chromosome have a highly significant effect on female's fecundity. There was, however, no significant correlation between *P1* and fecundity. These results suggest different genetic bases for the two phenomena. At present, we have no candidate genes for the factors that influence the female's ability to make differential use of sperm, but likely candidates are protein receptors in the female's reproductive tract where sperm is stored.

A recent study of sperm competition in *Drosophila* has shown that the chances of a male fertilizing the female's egg are dependent on both male and female genotypes, suggesting that female × male interactions play an important role in sperm precedence (Clark *et al.*, 1999). Then, it becomes interesting to note that while male offence ability and the female's tendency to promote displacement were negatively correlated, a positive and marginally significant correlation was found between male defence ability and the female's tendency to promote displacement. Any conclusion drawn from these results should be treated with caution due to the limited number of lines analysed and the lack of significance of the correlations. However, the trends detected are worth mentioning since they predict that females from lines where males are weak offenders should be promoters of sperm displacement and vice versa. It is possible that by evolving mating strategies that counteract the direction imposed by males, females may avoid deleterious effects (i.e. inbreeding, reduced viability) that may arise if males gain control over the females' mating strategies (Chapman *et al.*, 1995; Rice, 1996, 1998; Markow, 1997; Parker & Partridge, 1998; Clark *et al.*, 1999).

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