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Genetic differences in mating success and female choice in seaweed flies (*Coelopa frigida*)

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An association is described in seaweed flies, *Coelopa frigida*, between the genotype at the alcohol dehydrogenase (*Adh*) locus and mating success in pairwise mating trials. Significantly higher mating success was observed in females that carried the *Adh-C* allele, but no association was observed between *Adh* genotype and male mating success. There was heterogeneity in the success of different combinations of males and females, but only when the female lacked the *C* allele. Analyses of video recordings indicated that *C*-bearing females spent longer mounted by males and that they less frequently rejected males. Evidence is presented for mate discrimination by females not carrying a *C* allele. The significance of there being genetic differences in both mating success and in female discrimination are discussed in the context of previous results on mating behaviour in natural populations.

INTRODUCTION

Darwin's theory of sexual selection (Darwin, 1871) proposed that during the mating process males compete with one another for females, and females choose between the available males. The evidence for male–male competition is convincing in a wide diversity of species, but it is only during the last decade that equivalent evidence has been found for female choice. Female widowbirds (Andersson, 1982), bowerbirds (Borgia *et al.*, 1985), sedge warblers (Catchpole, 1987), zebra finches (Burley, 1986) and pied flycatchers (Read, 1986) all exercise choice on the basis of some male attribute. Examples from other animal groups include sticklebacks (Semler, 1971), frogs (Ryan, 1983) and several species of insects (see Thornhill and Alcock, 1983).

The demonstration that female choice occurs is only the first step in understanding how sexual selection operates over evolutionary time. This problem has been tackled from a theoretical point of view in the models of Fisher (1930), O'Donald (1980), Lande (1981) and Kirkpatrick (1982). It is clear that for female choice to evolve and be maintained, both the preference itself and the preferred character should be inherited. Furthermore, there should be some advantage to females being choosy which may be manifest as increased fecundity, or greater survival of the progeny. Bearing on this latter point are experiments showing that the exercise of choice results in increased reproductive success in zebra finches (Burley, 1986), tungara frogs (Ryan, 1983) and seaweed flies (Crocker and Day, 1987). Mate choice may also lead to the production of progeny better able to survive under competitive conditions as in fruit flies (Partridge, 1980) and seaweed flies (Crocker and Day, 1987). The experimental support for female choice being inherited is more limited. Only in the two-spot ladybird beetle, *Adalia bipunctata*, does direct evidence exist for a polymorphism with a genetic basis (Majerus *et al.*, 1982, 1986; O'Donald and Majerus, 1985; Majerus, 1986), although convincing indirect evidence has been obtained in *Drosophila melanogaster* (Heisler, 1984) and the Trinidad guppy, *Poecilia reticulata* (Breden and Stoner, 1987). We report here an association in seaweed flies between mating success, female choice and the possession of a particular allele at an enzyme-determining locus.

All populations of the seaweed fly, *Coelopa frigida*, are polymorphic for a large inversion on chromosome I (Butlin *et al.*, 1982*a*; Day *et al.*, 1983). This chromosomal rearrangement, which involves over 10 per cent of the genome (Aziz, 1975; Day *et al.*, 1982), has profound influences on the flies' biology. The two alternative forms (α and β) are associated with differences in development time (Day *et al.*, 1980), adult size (Butlin *et*

al., 1982*b*), adult longevity (Butlin and Day, 1985) and larval survival (Butlin *et al.*, 1984). There is also differential male mating success between genotypes (Butlin *et al.*, 1982*b*; Day *et al.*, 1987) and populations exhibit negative assortative mating with respect to the $\alpha\beta$ inversion (Day and Butlin, 1987). A recent analysis of mating (Crocker and Day, 1987) suggested that female seaweed flies might be choosing their mates on the basis of inversion genotype, and that there might, in addition, be an element of male choice.

This study by Crocker and Day used a laboratory population that had been intentionally bred to have reduced genetic variation. In particular, the population only included individuals possessing *B* and *D* alleles at the alcohol dehydrogenase locus (*Adh*). This locus is known to be associated with the $\alpha\beta$ inversion system such that *BB* homozygotes are always $\alpha\alpha$ homokaryotypes, and *DD*'s are always $\beta\beta$'s (Day *et al.*, 1982). A third allele, *Adh-C*, is also commonly found in natural populations. Preliminary mating tests involving females carrying at least one *C* allele suggested that in these cases mating was random. This admitted the possibility that *BB*, *BD* and *DD* animals were mating discriminately, but that *BC*, *CC*, and *CD* females did not exhibit mate choice. Here we report evidence in support of this interpretation, and suggest that, as in ladybirds, there are genetic differences in female choice. The data derive from pairwise mating trials in which the results of Crocker and Day, (1987) are extended to include the *Adh-C* allele. Observations of mating behaviour provide direct evidence that females not carrying the *Adh-C* allele mate in a discriminating fashion.

METHODS

Animals were collected from a natural population at St. Mary's Island on the north-east coast of England and were used in experiments within two generations of being collected. The maintenance of cultures in the laboratory has been described by Day and Buckley (1980). Adults were collected shortly after eclosion and males and females stored separately at 4°C. When required for pair trials flies were transferred to fresh seaweed (*Fucus serratus* and *F. vesiculosus*) at 29°C overnight. For video recording, animals were left for a further two days before use. Preliminary experiments showed that sex-isolation for one day resulted in the appropriate level of sexual activity in 5-h mating trials (appropriate, that is, for analytical purposes), but that a longer period of sex-starvation was necessary prior to video trials lasting only 45 min. Checks for virginity indicated that in no case had any female been fertilized before the start of trials.

The experimental procedures for pairwise mating trials and for the video recording of mating behaviour were those of Crocker and Day (1987) and Day *et al.* (1988), with the minor modification that one female and three males were observed in each trial. Males were distinguishably marked with small spots of paint applied to the dorsal surface of the thorax. Just over 100 trials were conducted each using different animals. On the completion of trials, animals were stored at −25°C and subsequently scored for their genotype at the alcohol dehydrogenase (*Adh*) locus using starch gel electrophoresis (Butlin *et al.*, 1982*a*).

RESULTS

Mating success in pair trials

Virgin flies were paired at random and the mating success of each pair assessed by whether or not they produced larvae. After the trial the *Adh* genotypes of both adults were determined. The mating success of males, regardless of the females with which they were paired, revealed no heterogeneity between genotypes (table 1). In contrast there were differences in the mating success of females. *BC*, *CC* and *CD* females were the most successful, and if the data from these three genotypes are pooled, there is a highly significant difference between the *C*-bearing genotypes and the combined non-*C*'s ($\chi^2_1 = 11{\cdot}71$; $P < 0{\cdot}001$). A similar comparison of *C* and non-*C* males reveals no difference ($\chi^2_1 = 0{\cdot}48$; $P = 0{\cdot}49$). It appears that possession of an *Adh-C* allele is associated with greater mating success in females, but not males.

Is the success of males (or females) dependent on the genotypes of the two animals considered together? The success of each male genotype generally did not vary with differing females, although *DD* males were an exception to this (table 2). When male *C*'s were pooled and non-*C*'s were pooled, the two sets of data were similar. The reciprocal comparison yielded a strikingly different result. While the success of *BC*, *CD* and *CC* females did not vary with the male genotype, there was the strong suggestion of heterogeneity between *BB*, *BD* and *DD* females. Furthermore, examination of the pooled data indicates that success was homogeneous with *C*-bearing genotypes, but heterogeneous with non-*C* females. Although non-*C* females are convincingly heterogeneous,

Table 1 Mating success of different genotypes in pair trials

	Per cent mating success *Adh* genotype							
	BB	*BC*	*BD*	*CC*	*CD*	*DD*	χ^2_5	*P*
Males	65·1	57·1	58·3	43·8	56·7	56·6	3·31	0·65
No. of trials	(83)	(42)	(503)	(16)	(90)	(228)		
Females	61·0	64·8	53·8	69·2	74·6	52·1	15·41	0·009
No. of trials	(105)	(91)	(513)	(13)	(63)	(163)		

χ^2 values (in this and all subsequent tests) were calculated from the original numbers of succesful and unsuccessful pairs.

Table 2 Mating success of different genotypic combinations of males and females

	Per cent mating success of males when paired with females of the following genotypes								
Male genotype	*BD*	*BB*	*DD*	*BC*	*CD*	*CC*	χ^2	df	*P*
BD	57·1 (261)	50·0 (58)	53·2 (77)	53·7 (41)	63·6 (22)	66·7 (6)	1·78	4	0·78
BB	56·8 (37)	77·4 (31)	60·0 (10)	— (0)	— (2)	— (0)	3·33	2	0·19
DD	51·3 (119)	56·3 (16)	32·6 (43)	78·6 (14)	75·0 (7)	— (1)	10·11	3	0·018
BC	55·6 (18)	— (0)	[66·7 (6)	— (1)	— (2)	— (0)]	FET*		0·90
CD	35·3 (34)	— (1)	— (4)	57·1 (14)	— (3)	— (1)	1·95	1	0·16
CC	20·0 (10)	— (0)	[— (1)	— (0)	— (0)	— (0)]	FET*		0·55
Non-*C*'s	55·4 (417)	59·0 (105)	46·9 (130)	60·0 (55)	61·3 (31)	57·1 (7)	5·20	4	0·27
C's	38·7 (62)	— (1)	54·5 (11)	60·0 (15)	— (5)	— (1)	2·75	2	0·25
	Per cent mating success of females when paired with males of the following genotypes								
Female genotype	*BD*	*BB*	*DD*	*BC*	*CD*	*CC*	χ^2	df	*P*
BD	57·1 (261)	56·8 (37)	51·3 (119)	55·6 (18)	35·3 (34)	20·0 (10)	10·80	5	0·056
BB	50·0 (58)	80·0 (30)	56·3 (16)	— (1)	— (1)	— (0)	7·48	2	0·024
DD	53·2 ((7)	60·0 (10)	32·6 (43)	66·7 (6)	25·0 (4)	— (1)	5·49	2	0·064
BC	53·7 (41)	— (1)	78·6 (14)	— (1)	57·1 (14)	— (0)	2·72	2	0·26
CD	63·6 (22)	[— (2)	42·9 (7)	— (2)	— (3)	— (0)]	FET*		0·81
CC	71·4 (7)	— (0)	[— (1)	— (0)	— (1)	— (0)]	FET*		0·33
Non-*C*'s	55·3 (396)	66·2 (77)	47·2 (178)	56·0 (25)	35·9 (39)	27·3 (11)	16·26	5	0·006
C's	58·6 (70)	— (3)	63·6 (22)	— (3)	61·1 (18)	— (0)	0·19	2	0·91

* When sample sizes (given in round brackets) were very low, trials have been pooled as indicated by square brackets, and a Fisher's exact test (FET) performed on the data.

the statistical test for homogeneity of *C*-females is not very powerful, since a large proportion of these females were paired with a single male genotype (*BD*). These results should not, therefore, be taken as strong evidence for the mating success of *C*-females being independent of male genotype. In passing we may note that the patterns of non-randomness shown by *BB*, *BD* and *DD* females do not appear to be the same, a point that will be considered in a later section.

The opportunity for insemination

Clearly, before a pair of flies can achieve a successful mating, the male must mount the female and remain mounted long enough for insemination. In order to study various aspects of mounting, trials were conducted with a single female and three males. The behaviour of animals was recorded for 45 minutes and the interactions quantified during slow play-back of the tapes. It should be noted throughout this section that none of the data were normally distributed and neither did any transformation render them so. The data have therefore been analysed non-parametrically using either the Mann-Whitney U-test, from which the normal deviate was computed, or the Kruskal-Wallis test in which H is distributed as χ^2 with five degrees of freedom (since six genotypes were being compared).

Table 3 Comparison of female genotypes with respect to various aspects of mounting

	Female genotype						Pooled *C*'s (*BC*, *CC* and *CD*)	Pooled non-*C*'s (*BB*, *BD* and *DD*)
	BB	*BC*	*BD*	*CC*	*CD*	*DD*		
Mean time to first mount (minutes) (s.e.)	2·6 (1·3)	2·4 (1·8)	1·7 (0·5)	3·9 (2·4)	0·8 (0·3)	3·8 (2·0)	2·4 (1·0)	2·9 (0·9)
Mean number of mountings per female (s.e.)	18·0 (3·9)	30·1 (5·9)	16·7 (1·8)	12·7 (4·9)	29·3 (5·1)	16·9 (1·8)	24·8 (3·5)	16·9 (1·2)
Mean duration of mounts (seconds) (s.e.)	28·9 (6·3)	37·5 (4·8)	37·6 (2·9)	56·7 (7·7)	25·8 (4·4)	35·2 (4·3)	36·9 (3·4)	36·1 (2·2)
Number of mountings	14	22	85	10	19	48	51	147
Per cent of trial spent mounted (s.e.)	28·8 (11·1)	37·7 (5·9)	23·7 03·2)	32·5 (8·5)	29·8 (5·4)	20·6 (3·4)	33·8 (3·6)	23·2 (2·4)
Number of females	5	10	35	7	7	20	24	50

The data are presented as means averaged over all females of a given genotype. For the calculation of mounting duration, mountings followed by an immediate female rejection or male dismount were excluded. In this case means derive from averages of extended mountings of a given female genotype.

The time elapsed from the beginning of the trial to the first mounting was measured (see table 3). There were no significant differences between any of the female genotypes, nor were the pooled *C*'s different from the pooled non-*C*'s ($U = 532$, $d = 0{\cdot}79$; $P = 0{\cdot}43$). However, the vast majority of females were mounted within the first 60 seconds of trials, a rapidity that is probably a consequence of the long period of sex-starvation to which all animals were subjected. If the animals had been less sex-starved it is possible that differences in mounting time might have been observed (but far fewer data on mountings would have been available for analysis).

There were significant differences in the mean number of times each female was mounted ($H = 12{\cdot}17$; $P = 0{\cdot}033$), but only a small difference between *C*'s and non-*C*'s ($U = 560$, $d = 1{\cdot}77$; $P = 0{\cdot}009$). Nevertheless, there is a strong suggestion that *BC*'s and *CD*'s were mounted more frequently than other genotypes. This result needs to be interpreted with care since many mounts resulted in an instant dismount by the male, thereby making the female available for almost immediate remounting. In contrast, a few mounts were of exceedingly long duration—indeed most of the trial—so that it was impossible for that female to be mounted many times.

For those mountings not terminated by immediate male dismounting nor female rejection, the mean duration of each mount was calculated. In this case there are significant differences between genotypes ($H = 12{\cdot}08$; $P = 0{\cdot}034$), but the differences are not associated with the possession of a *C* allele (comparing *C*'s with non-*C*'s gives $U = 3576$, $d = 0{\cdot}49$; $P = 0{\cdot}62$). The analysis suggests that *CC*'s are mounted for long duration, whereas the mountings of *BB* and *CD* females tend to be short.

The combined effects of all these aspects of mounting were explored by calculating the total time spent by females with a male mounted upon them. The three *C*-bearing genotypes all spent a larger proportion of the trials mounted than did *BB*'s, *BD*'s or *DD*'s (see table 3), and when combined, there is a highly significant difference between *C*'s, and non-*C*'s ($U = 418$, $d = 2{\cdot}59$; $P < 0{\cdot}01$).

The conclusion from this analysis is that there may well be greater opportunities for insemination of *C*-bearing females compared with non-*C*'s. Furthermore, we tentatively suggest that the three *C* genotypes gain their opportunities in slightly different ways. At one extreme *CD* females are mounted with little delay, subsequent mountings occur frequently but each is of short duration. In contrast *CC* individuals are mounted more slowly and less often, but each mounting is of long duration. These three attributes combine in their various ways to result in all three *C* genotypes

being mounted for about 15 minutes of the 45 minute trial. *BB*'s, *BD*'s and particularly *DD*'s are mounted for a distinctly shorter time.

Similar analyses were also performed on the six male genotypes but the data are not presented in detail here. Suffice it to report that no differences were observed in the times to first mount, total number of mounts, mean duration of each mount, or in the total time spent mounted. This lack of any association between male genotype and various aspects of mounting is consistent with the similarity in mating success of males observed in pair trials.

Male rejection of females

A frequent consequence of a mounting is that the male dismounts with no apparent female rejection response. If for some reason certain females are more "attractive" to males, those females should be rejected less often. There are in fact no differences between female genotypes in this respect ($H = 3{\cdot}23$; $P = 0{\cdot}67$), although *BB* and *BC* females were rejected slightly less frequently than other genotypes. There were also no differences in the rejection rate by different male genotypes ($H = 4{\cdot}6$; $P = 0{\cdot}47$). There appears to be no association between genetic variation at the *Adh* locus and male rejection.

Female rejection of males

The female rejection response involving kicking, wing lifting, abdominal curling and eventually lateral rolling of the complete body has been described elsewhere (Day *et al.*, 1988). We now enquired whether any relationship exists between the *Adh* locus and female rejection, and if so, whether it could contribute to the pattern of mating success observed in pair trials.

When all mountings were considered (table 4), there were no differences between female genotypes in their rejection rate ($H = 6{\cdot}75$; $P = 0{\cdot}24$), although *DD*'s showed a distinctly higher rate of rejection than other genotypes. However, a review of the original data suggested that a few individual animals were contributing disproportionately to the number of mounts (males) and the number of rejections (females). Furthermore, the behaviour of many females changed during the trial (see below). In an attempt to remove these effects, only the first interactions between each male and female were scored. The data (table 4) reveal large differences between females ($\chi^2_5 = 17{\cdot}3$; $P = 0{\cdot}004$), with *BC* females rejecting half as often, and *DD*'s rejecting twice as often, as the remaining genotypes. Overall, the non-*C*-bearing genotypes rejected about twice as often as *C*'s ($\chi^2_1 = 6{\cdot}3$; $P = 0{\cdot}012$), although this difference is largely due to *DD* females.

Having demonstrated differences in the willingness to mate, we may now ask if some, but not all, females are also "fussy", or discriminating in which males they reject. If females are classified as consistent rejectors—rejecting each different male who mounted—consistent acceptors—accepting every male who mounted—or mixed rejectors/acceptors (*i.e.*, possible discriminators), a further difference between genotypes is revealed. One third of *C* genotypes (8/24) exhibited mate discrimination while over a half (29/54) of non-*C*'s were discriminating. Collectively *BB*, *BD* and *DD*

Table 4 Relationships between female genotype and female rejection

Female genotype	Per cent of total mounts ending in female rejection (s.e.)	Per cent of initial mounts ending in female rejection (number of females)	Number of females exhibiting		
			Consistent rejection	Some rejections, some acceptances	Consistent acceptance
BB	2·6 (1·7)	30·8 (13)	0	3	2
BC	2·5 (1·4)	12·0 (25)	0	4	6
BD	4·6 (1·2)	28·6 (84)	3	16	11
CC	2·6 (2·1)	28·6 (14)	0	2	5
CD	2·8 (2·2)	22·7 (22)	0	2	5
DD	14·3 (4·2)	53·8 (52)	5	10	4
Pooled *C*'s	2·6 (1·0)	19·7 (61)	0	8	16
Pooled Non-*C*'s	7·7 (1·7)	37·6 (149)	8	29	17
Tests for heterogeneity between *C*'s andNon'*C*'s	d = 1·42 $P = 0{\cdot}16$	$\chi^2_1 = 6{\cdot}3$ $P = 0{\cdot}012$		$\chi^2_2 = 9{\cdot}0$ $P = 0{\cdot}007$	

females were significantly more choosy than females carrying a *C* allele (table 4).

Is there any evidence that the pattern of discrimination varies between female genotypes? In this further breakdown of the data, the only informative genotypes were *BD* and *DD*—both putative discriminators. *BD* females rejected 33 per cent (sample size 18) of *BD* males, but 75 per cent (8) of *DD* males. In contrast, *DD* females rejected 71 per cent (7) of *BD* males but none of the three *DD* males. In spite of the small sample sizes there is significant heterogeneity in rejection ($\chi_1^2 = 8{\cdot}2$; $P = 0{\cdot}042$: Lewontin and Felsenstein (1965) have shown that this test for heterogeneity is valid even with exceedingly low expectations). It appears, then, that at least *BD* and *DD* females are discriminating in who they reject, and if this rejection influences mating success, it predicts there should be positively assortative mating with respect to the *Adh* locus.

This evidence for female discrimination comes from considering only the initial mount by each male. If the sequence of rejections and acceptances during the course of trials is examined, further indication of discrimination is apparent. In table 5, details of five such sequences are shown. In trial 83 there is clear evidence that the *BB* female repeatedly accepted the *CC* and *CD* males but rejected the *BD* male. Such consistent behaviour was not typical of most females. It was more common for a female initially to reject a male but subsequently accept him (*e.g.*, trial 49). The generality of this phenomenon is seen in the overall rejection rate being very much lower than the rate of rejection following initial mounts (table 4). Occasionally the female seemed to get fed up with mating, and began rejecting males she had earlier accepted (*e.g.*, trial 41).

Trials 83, 49 and 41 involved non-*C* bearing females who showed both acceptance and rejection. While most *C*-bearing females were consistent acceptors, a few did reject as well. Trials 21 and 100 are two examples. In these, and the other six similar trials, individual males are not repeatedly rejected or accepted. In other words acceptance or rejection does not seem to be related to the males' genotype. We have tested more formally the proposition that *C* females who both accept and reject are doing so regardless of which male has mounted, whereas non-*C* females are genuinely discriminating between males. 2×3 tables were produced for each trial summing the number of acceptances and rejections for each of the three males. For example, trial 83 yields the table:

	Male 1	Male 2	Male 3
Acceptances	5	6	0
Rejections	0	0	5

whereas for trial 21:

	Male 1	Male 2	Male 3
Acceptances	2	3	3
Rejections	3	2	5

It is obvious that the statistical heterogeneity in the first contingency table is far greater than in the second. For each of the eight such tables from *C* females, and 19 for non-*C* females, the probability of obtaining such a set of data was calculated using

Table 5 Sequence of female rejection/acceptance in individual trials

Trial 83	female: *BB*; male 1: *CC*; male 2: *CD*; male 3: *BD* 1a, 3r, 2a, 1a, 3r, 2a, 2a, 2a, 3r, 3r, 1a, 1a, 2a, 1a, 3r, 2a.
Trial 49	female: *DD*; male 1: *BD*; male 2: *BB*; male 3: *BC* 2r, 3a, 2a, 2a, 2r, 1r, 1r, 2a, 1r, 3a.
Trial 41	female: *BD*; male 1: *BD*; male 2: *CD*; male 3: *BD* 1a, 3a, 3a, 3a, 3a, 2r, 3a, 3a, 2r, 3r, 1r, 3a, 2r, 3a.
Trial 21	female: *CD*; male 1: *BC*; male 2: *BC*; male 3: *CD* 3a, 1a, 1a, 2a, 2r, 3r, 2r, 1r, 1r, 3r, 3r, 2a, 3a, 2a, 3r, 3a, 1r, 3r.
Trial 100	female: *BC*; male 1: *BB*; male 2: *BD*; male 3: *CD* 1a, 3r, 3a, 3r, 3r, 3a, 3r, 3r, 3a, 3a, 3r, 3r, 3r, 3r, 3a, 3a, 3r, 1r, 3r, 3r, 2r, 2r, 3r, 3a, 3r, 2r, 3a, 2a, 2a, 2r, 2a, 2r, 2r, 3a, 3a, 1a, 2a.

Rejection is represented by "r", and acceptance by "a". Trial 83 began with male 1 being accepted, then male 3 was rejected, then male 2 accepted and so on. Male dismounts have been omitted from these sequences.

Fisher's exact test. It must be pointed out that most of the trials were not nearly as extreme as the two examples given above. Nevertheless, when the probabilities are combined, using the method of Fisher (1934), none of the *C* genotypes is heterogeneous (*BC*: $\chi^2_8 = 2{\cdot}2$, $P = 0{\cdot}98$; *CD*: $\chi^2_4 = 1{\cdot}7$, $P = 0{\cdot}79$; *CC*: $\chi^2_2 = 0{\cdot}6$, $P = 0{\cdot}73$), whereas the non-*C*'s are (*BB*: $\chi^2_6 = 20{\cdot}6$, $P = 0{\cdot}002$; *BD*: $\chi^2_{16} = 26{\cdot}0$, $P = 0{\cdot}05$; *DD*: $\chi^2_{16} = 52{\cdot}2$, $P \ll 0{\cdot}001$). The combined heterogeneity with non-*C* females is very considerably greater than with *C*'s (non-*C*'s: $\chi^2_{38} = 98{\cdot}7$, $P \ll 0{\cdot}001$; *C*'s: $\chi^2_{14} = 4{\cdot}4$, $P = 0{\cdot}99$). This provides yet more evidence that for those females exhibiting mixed acceptance/rejection behaviour, the non-*C*'s are genuinely discriminating, whereas the *C*'s are not.

We consider the critical observation in many of the trials to be that females rejected and accepted males in successive mounts; they were physiologically able to carry out both types of response. For *C*-females this behaviour was random, but for other genotypes it was highly discriminating. It seems as though both willingness to mate, and lack of mate discrimination is conferred by the *C* allele, or by alleles in coupling at closely linked loci.

DISCUSSION

It is widely assumed that female choice is genetically determined yet direct experimental support only exists for *Adalia bipunctata* (Majerus *et al.*, 1986). In the two-spot ladybird a single dominant gene appears to be responsible for female preference for melanic males, with homozygous recessive females mating randomly. Preference for non-melanic males has not been observed. The results reported here provide further evidence that female choice has a genetic basis. Seaweed flies possessing an *Adh-C* allele exhibit no mate discrimination whereas other genotypes appear to exercise mate choice. We are still a long way from identifying the gene or genes responsible for this behaviour, but there is currently no reason at all to believe the alcohol dehydrogenase locus itself is crucial. The *Adh* locus is associated with a large chromosomal inversion and any of the 200 or more genes in this inversion could be responsible. What does appear to be true, however, is that whatever genes determine female choice they are genetically linked to the genes determining the male character being chosen—namely genotype at the *Adh* locus, or loci closely linked to it. Such association between preference and preferred character may have been of significance during the evolution of female choice.

There is still some doubt over the tightness of this association. While recombination is exceedingly low between the α and β forms of the inversion, *Adh-C*, as identified by starch gel electrophoresis, can be found on either inversion (Day *et al.*, 1982). It is clearly of consequence to know whether one or both inversion sequences carry the allele for non-preference. Whatever the outcome, one could expect the genes responsible for female preference to recombine with at least one of the inversion types. Why then do we observe association between the loci for *Adh* and preference? Possibly the two loci are exceedingly tightly linked, or there may be selective reasons why these, and perhaps other loci, should remain in linkage disequilibrium.

Regardless of the solution to this genetical problem, we can make a prediction concerning behaviour. If the non-preference allele is located on only one of the inversions, it should be possible to make a much more clear-cut distinction between choosy and non-choosy females. The category of non-dicriminators as presently identified may well include females with both types of behaviour. The genetic determination of female choice is being studied further.

Consider next the patterns of mating success. Several factors, both genetic and non-genetic are known to be associated with mating success in seaweed flies. These include female choice on the basis of the $\alpha\beta$ inversion, male choice on the basis of size (of which this same inversion is a major determinant), male-male competition and the forced mating of unwilling females (*i.e.*, rape). In such a complex situation it is to be expected that a complete understanding of mating will not readily be achieved. The results presented here are not obviously consistent with previous data. For example, the least successful males in conditions of mass mating were *Adh-BB* homozygotes, and *Adh-CD*'s were startlingly successful (Day *et al.*, 1987). In contrast, pair trials conducted in small enclosures revealed no significant differences in male success, and if anything, *BB*'s were marginally more successful than other genotypes (see Table 1). In mass matings female success was homogeneous whereas heterogeneity was observed between genotypes among the results reported here. Furthermore, the pattern of mating was predominantly negatively assortative in the results of Day and Butlin (1987) whereas in the present

experiments the flies tended to exhibit positive assortment.

At the moment we can only speculate on the reasons for these apparent discrepancies. Clearly the mating conditions were not identical. In pair trials male–male competition is absent and females may find it difficult to exercise choice when closeted with a large male. There were also differences in the levels of inbreeding, genotypic frequencies, sex starvation and encounter rates, all of which could affect mating success. We certainly believe the previous findings of negative assortment to be valid and indeed R. K. Butlin (personal communication) has pointed out that there are several indications that it is also operating under the experimental conditions used in the present study. The problem will be to disentangle the various components of mating behaviour and assess their genetic and evolutionary effects. We should not be surprised if it is the balance between female choice and male choice that largely determines the pattern of mating success. Needless to say, we are actively pursuing this problem.

There is evidence that the inversion polymorphism has been stable in natural populations for almost two decades (Kelsey, 1969), and the uniformity in inversion frequencies over northern Europe suggests the polymorphism may be of considerable antiquity (Butlin *et al.*, 1982; Day *et al.*, 1983). What are the selective forces maintaining this stability, and in particular, why is there a polymorphism for genes affecting female choice? The results of Crocker and Day (1987) suggested an advantage to being discriminating. When animals were given the opportunity to exercise mate choice, a larger proportion of females were mated and their progeny had better survival, compared to animals given no choice. Higher fecundity and superior progeny fitness obviously constitute a selective advantage for mate choice. What is the evolutionary point of being non-discriminating? Under conditions of low adult density, females may encounter rather few males and non-choosy females may then be more likely to breed than those who are choosy. Results to be published elsewhere indicate that flies carrying an *Adh-C* allele do indeed enjoy a selective advantage over other genotypes when the encounter rate is low (H, Sawyer, K. Kristou, A. Brown and T. Day, unpublished results). In seaweed flies there is a genetic polymorphism for mate discrimination that could be maintained in a state of balance, with discriminating animals being at an advantage due to natural selection at high population densities, but at low densities being subject to adverse sexual selection.

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