Differential mating success in populations of seaweed flies (*Coelopa frigida*)

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The reproductive success of seaweed flies (*Coelopa frigida*) was studied in natural populations. The sizes of females, both mothers and those not producing eggs, as well as of the males available to be fathers, were estimated from their wing lengths. The animals were also classified for their genotype at the alcohol dehydrogenase (*Adh*) locus—a polymorphic locus tightly associated with a large chromosomal inversion. The fathers' genotypes were inferred from the genotypes of the mother and her offspring. Males of different *Adh* genotypes had different reproductive success. Reproductive success of genotypes was negatively correlated with the mean sizes of males. Differences in the reproductive success of females could be accounted for by their differential egg laying capacity, and is probably not a consequence of their mating behaviour. The incidence of progeny with mixed paternity varied from 20 per cent initially, to about 65 per cent when the animals had been mating for 6 days. It appears from previous work that large males are more successful in laboratory trials, yet under natural conditions genotypes associated with large male size have low mating success. Ways of reconciling these results and their relevance to understanding the inversion polymorphisms are discussed.

INTRODUCTION

For 125 years Darwin's ideas of natural selection have dominated the thinking of students of evolution (Darwin, 1859). It is only in the last 10–20 years that the other major Darwinian force of evolution—sexual selection (Darwin, 1871)—has provoked experimental biologists in a comparable way. There are several recent reviews that bear witness to this resurgence of interest (Campbell, 1972; Blum and Blum, 1979; O'Donald, 1980; Bateson, 1983). It has become clear that sexual selection may be implicated in the maintenance of many genetic polymorphisms, for example, inversion polymorphism in *D. pseudoobscura* (Spiess *et al.*, 1966) and *D. persimilis* (Spiess and Spiess, 1967), colour polymorphism in ladybirds (O'Donald and Majerus, 1984), arctic skuas (O'Donald, 1983) and sticklebacks (Semler, 1971).

The sexual component of selection in systems of this type may be much greater than other components such as viability and fecundity (Prout, 1971). No study of selective pressures influencing a polymorphic system can be complete, therefore, without a consideration of sexual selection. Furthermore, polymorphic systems have a great poten-

tial for investigating the operation of sexual selection, and its interaction with other components of selection. The pioneering work of O'Donald and his associates on *Adalia bipunctata* (O'Donald and Majerus, 1985) demonstrates that results of general importance can be obtained.

Previous work on the dipteran seaweed fly, *Coelopa frigida*, has shown that a variety of selective pressures act on an inversion polymorphism on chromosome I. Karyotypes differ in development time (Day *et al.*, 1980), adult body size (Butlin *et al.*, 1982a), viability (Butlin *et al.*, 1984), longevity and fecundity (Butlin and Day, 1985). In laboratory tests where a female was able to mate with either a large or a small male, the large male sired her progeny in about 80 per cent of trials (Butlin *et al.*, 1982a). The karyotypes of the males did not influence this result but in natural populations the association between karyotypes and male size was expected to produce an association between karyotype and male mating success. We have attempted to demonstrate such an association in nearly natural conditions and have found marked differences in male mating success. However, these differences do not appear to be associated with large male size.

GENERAL MATERIALS AND METHODS

Animals were obtained from natural populations at two sites on the north-east coast of England: St. Mary's Island (Ordnance Survey map reference NZ 350753) and Whitburn (NZ 408614). Adults were collected using an insect aspirator, and larvae sampled using the "pre-adult" method described by Butlin *et al.* (1982a). The size of adults was estimated from the length of their wings (Butlin *et al.*, 1982b). Their genotype at the alcohol dehydrogenase locus (*Adh*)—used as a marker for the chromosome I inversion (Day *et al.*, 1982)—was determined by starch gel electrophoresis (Butlin *et al.*, 1982a), with the minor modification that 3-(4-,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide (MTT) was used in the staining mixture instead of NBT.

Pilot experiment

The most direct way of studying mating success in wild populations of insects is to capture mating pairs. In seaweed flies mating usually takes less than 60 seconds, and animals are only rarely seen together as pairs. An additional problem is that mating occurs beneath the surface of the seaweed deposit, and disturbance of the weed results in the animals taking flight. In practice it has not proved possible to observe directly the mating behaviour of flies in their natural habitat. A less direct approach was necessary to study mating success. We carried out a pilot study to test the feasibility of identifying the genotypes of fathers inferred from the analysis of their progeny, and then comparing them with the genotypes of males available to be fathers. This technique has the advantage of measuring only successful copulations.

Males and females from a seaweed bed at St. Mary's Island were separated immediately after collection, 320 females placed individually in containers with fresh seaweed. The *Adh* genotype of each female that laid fertile eggs was determined, as were the genotypes of her progeny after they had grown to a sufficient size. The number of progeny examined was usually between 8 and 12. Knowing the genotypes of the mother and her progeny allows us to infer the genotype of the father. In addition the genotypes and sizes of females that did not lay eggs, and of the males present in the original sample, were assessed.

Some females collected did not lay eggs (110), laid infertile eggs (172), or laid too few fertile eggs to infer unambiguously the father's genotype (5). Two females produced progeny that could not be accounted for in terms of a single male parent and so must have resulted from multiple insemination. In addition one female had been mated by a male with a very rare genotype (*Adh-DE*). In this, and in all subsequent experiments, animals carrying the rare *Adh-A* or *E* alleles, as well as *CC* homozygotes, have been omitted from the analysis. This amounts to an omission of less than 1 per cent of the data. From the original 320 females, we obtained information on 30 matings.

The genotypes of mothers, fathers, females and males, and the sizes of males, are given in table 1. The mothers were a random sample of the females present, but the inferred fathers are significantly different from the males present, at least with respect to their *Adh* genotypes. This small sample of only 30 matings also provides no evidence that large males are more successful than small ones. Indeed, nearly half the matings seem to have involved the genotypic class of smallest mean size.

Table 1 Comparison of *Adh* genotype frequencies in mothers, fathers, females and males (pilot experiment)

	Adh Genotype frequencies (%)					
	BB	BC	BD	CD	DD	Sample size
Females present	10·4	6·6	44·3	14·8	24·0	183
Mothers	16·1	6·5	41·9	16·1	19·4	30
Males present	5·1	6·7	45·6	12·3	30·3	195
Inferred fathers	3·3	3·3	20·0	26·7	46·7	30
Mean (and range) of sizes of males	8·80 (6·34–10·88)	7·70 (5·89–9·29)	8·20 (6·12–10·20)	7·41 (6·12–9·97)	6·88 (5·44–8·61)	

There is no significant difference between genotypes of mothers, and females available to be mothers ($\chi_3^2 = 1 \cdot 10$, $p = 0 \cdot 78$).

The inferred genotypes of fathers are significantly different from those of males available to be fathers ($\chi_2^2 = 9 \cdot 50$, $p = 0 \cdot 009$). (Here and elsewhere χ^2 values have been calculated using numbers rather than frequencies).

The sizes of males are expressed in graticule units (1 unit = 0·64 mm). Mean sizes differ significantly between genotypes ($F_{4,194} = 23 \cdot 0$ $p < 0 \cdot 001$).

However, the main conclusion from this pilot experiment was that it was an extremely laborious and inefficient method of studying mating. A large proportion of the original females were infertile, presumably because of virginity or senility. In addition, the experiment can be criticised on the grounds that the males collected at the time of sampling were not necessarily representative of those available to be fathers at the time of mating. Differences in the longevity of males (Butlin and Day, 1985), and in particular, differences in the hatch sequence of the various genotypes (Day *et al.*, 1980), result in genotype frequencies changing with time. Multiple insemination and the incorrect inference of the father's genotype (due to the limited number of progeny being scored) are further potential sources of inaccuracy.

Main experiment

In an attempt to overcome at least some of these shortcomings a different method of sampling was used. Instead of adults, samples of larvae, and of the seaweed in which they were living, were collected. These were then maintained at room temperature (22°C) until the adults eclosed. They were left undisturbed to mate with whom they chose, and as often as they chose. After varying lengths of time, the adults were aspirated from the cages, and the males and females separated immediately. The males were frozen (−25°C) for subsequent measurement and genotyping, and the females individually allowed to lay eggs. Females and their progeny were analysed as before.

We report here the results from three sets of samples each with slight modifications of the basic experimental design. The essential features of the three experiments were as follows:

Sample 1 Larvae were collected from St. Mary's Island in November 1979 and a single sample of adults taken just after the time of peak eclosion.

Sample 2 Larvae were collected in three replicate cages from St. Mary's Island in September 1981. Adults from the first cage (A) were sampled 1 day after the start of eclosion; the second cage (B) was sampled 3 days later, and the third cage (C) after a further 2 days. Adult sizes were not measured.

Sample 3 Larvae were collected in four replicate cages from Whitburn in November 1982. Adults from cages A, B, C and D were aspirated 2, 4, 6 and 8 days respectively, after adults were first seen. All males and females were measured. 12 progeny were used to infer paternity whenever possible.

Mixed paternity

Early studies on mating in *Coelopa* suggested that if a female is presented with first one male and then a second, it is the second male that sires the progeny (Thompson, 1951; Burnet, 1961). However if the two males are offered simultaneously then in about 15 per cent of cases the progeny are of mixed paternity (Butlin *et al.*, 1982*a*). In the pilot study of wild caught flies, about 6 per cent (2/33) of females appeared to have been multiply inseminated.

The most reliable estimates of mixed paternity emerged from samples 3A, B, C and D in which the largest number of progeny had been studied (usually 12 animals). From table 2 it can be seen that the proportion of progeny sired by more than one male varied from 3–9 per cent. However, this must be an underestimate, since some progeny must have been interpreted as resulting from a single father when in fact there were contributions from more than one male. For example, if an *Adh-BB* mother had in fact been inseminated by both a *BB* and a *BD* male, then the inferred father would be a single *BD* male. In other words, we were unable to distinguish between sampling error causing a deviation from Mendelian expectations, and distortions due to sperm mixture. However, if we make some simple assumptions then we can attempt to correct for this type of error. The assumptions and general formulae used in these calculations are given in the Appendix.

A further correction incorporated into the calculation of the expectations is sampling error

Table 2 Frequency of mixed paternity

Sample	Observed incidence of mixed paternity (%)	Calculated incidence of multiple insemination (per cent) (observed and undetected)
Pilot	6·3	40·3
1	4·8	32·2
2A	3·9	27·1
2B	3·9	32·2
2C	6·8	57·9
3A	3·3	18·6
3B	6·7	43·5
3C	9·1	64·4
3D	6·3	32·4

The "observed incidence" of mixed paternity is that proportion of broods found to possess three or more paternally derived alleles.

The "calculated incidence" is the estimated probability (per cent) of a female being inseminated by two males. (This is the value of *x* in the model described in the Appendix).

resulting from the limited number of progeny studied. We may by chance have failed to sample one genotype in the progeny and thereby incorrectly inferred the father's genotype. In practice this type of error is negligible (1–2 per cent) compared to that from mixed paternity. In general terms, the "corrected" expectations compensate for the under-estimation of homozygote frequencies, and the over-estimation of the frequencies of heterozygotes.

The calculated proportions of multipaternal families (whether detected as such or not) are given in table 2. It is clear from these data that multiple insemination is quite frequent; about 30–40 per cent of broods are of mixed paternity. Not surprisingly it increases with time. Sample 3A was taken first, and B, C and D at two day intervals thereafter. The same trend is seen in 2A, B and C. (Sample 3D was small—if 2 instead of 1 mixed mating had been observed, then the incidence would have been 64·7 per cent.)

Finally, these data indicate that the experimental technique grossly underestimated the frequency of mixed paternity. This must mean that the observed genotype frequencies among fathers are also distorted. For this reason the corrections outlined in the Appendix have been applied to generate modified expectations of paternal frequencies used in the next two sections.

Are fathers a random sample of the males available to be fathers?

There are two possible methods of analysing the data. The first involves calculating the sperm gene frequencies from the frequencies of genotypes in each brood. The frequency of paternally contributed *genes* can then be compared with the expected contribution calculated from the males available to be fathers. This is the approach used by Wilson (1981). An alternative method is to infer the father's genotype for each brood, and then to compare these *genotype* frequencies with those present in males available to be fathers. The first method is very sensitive to any viability differences between progeny individuals. The second method does not rely on the relative frequencies of genes in the progeny, but merely on their presence or absence. Since there is evidence for differences in viability among developing larvae (Collins, 1978; Butlin *et al.*, 1982b; 1984), we have used the second method. It should be pointed out that the conclusions to which we come are not dependent on which method of analysis is used.

The observed and expected genotype frequencies among fathers for each sample are given in table 3. It can be seen that there is a fairly general deficiency of fathers with the genotypes *BB*, *BC*, and *BD*, and an excess of fathers with *CD* and *DD* genotypes. The values of χ^2 given derived from comparisons between the observed numbers of fathers and the numbers expected on the assumption of random mating. These expectations incorporate the corrections for mixed paternity and for the limited numbers of progeny examined. In all but two samples for which a valid statistical test could be carried out there is a very highly significant departure from randomness.

There are, of course, many ways in which these data could depart from randomness. The critical question is whether there is consistency in the patterns of non-randomness. If each male genotype in each sample is assigned a "success" rating (the ratio of observed/expected genotype frequencies in fathers), and these ratings are ranked within samples, then the nine series of ranks can be compared. From Kendall's coefficient of concordance ($w = 0·768$, $p < 0·001$; see Sokal and Rohlf, 1981), it is clear that animals in the different samples are behaving in a homogeneous fashion.

We are particularly impressed by the consistent success of *CD* males as fathers (more than three times the expected), and the lack of success of *BB* males (about one quarter the expected).

These differences in genotype frequencies between inferred and expected fathers could be generated in several ways. They could result from real differences in mating success between genotypes, in which case they would probably be mediated by some phenotypic character associated with the chromosome I inversion. The obvious candidate in view of previous work (Butlin *et al.*, 1982a) would be size, but the inversion contains many genes, so that a wide range of possible characters exists. The differences could be a result of the variation in development time between genotypes (Day *et al.*, 1980) with no variation in mating success amongst mature males available at the time of mating. Finally, they could be an artefact of the procedures used to infer paternity and to calculate the expected distribution of fathers. The example given in the Appendix demonstrates that, despite the inferred high frequency of multiple insemination, the corrected expectations differ little from expectations based simply on the genotype frequencies amongst available males. We can envisage no way in which our methods of analysis could generate the large and consistent deviations observed.

Table 3 Comparison of observed and expected *Adh* genotype frequencies (percentages) among fathers

Sample		*Adh* Genotype					Sample size	χ^2	df	p
		BB	BC	BD	CD	DD				
Pilot	Obs.	3·3	3·3	20·0	26·7	46·7	30	7·52	2	0·023
	Exp.	4·0	5·7	51·7	13·0	25·7				
1	Obs.	0	5·0	45·0	30·0	20·0	20	2·77	2	0·250
	Exp.	6·4	10·4	41·1	7·9	34·2				
2A	Obs.	0	0	11·1	55·6	33·3	27	15·12	2	<0·001
	Exp.	3·2	3·6	52·2	12·4	28·5				
2B	Obs.	4·2	2·8	40·3	26·4	26·4	72	14·81	2	<0·001
	Exp.	0·7	1·1	72·5	8·9	16·8				
2C	Obs.	0	2·6	38·5	35·9	23·1	39	11·03	2	0·004
	Exp.	8·0	9·2	60·6	4·1	18·0				
3A	Obs.	3·2	3·2	46·0	17·5	30·2	63	3·86	2	0·145
	Exp.	4·2	8·1	58·8	10·7	18·1				
3B	Obs.	0	6·9	29·8	35·9	27·5	131	38·65	4	≪0·001
	Exp.	5·6	6·5	59·1	12·4	16·4				
3C	Obs.	0	3·3	36·1	21·3	39·3	61	23·75	3	<0·001
	Exp.	4·8	9·3	68·9	6·3	10·5				
3D	Obs.	0	26·7	33·3	13·3	26·7	12	No valid		
	Exp.	18·8	16·1	47·7	9·4	8·1		test		
Pooled nos.	Obs.	6	22	157	135	138	458	117·5	19	≪0·001
	Exp.	22·6	30·9	277·2	44·9	81·8				

The "observed" genotype frequencies are based on inferred paternity from the analysis of maternal and progeny genotypes. The "expected" genotype frequencies are based on the males available to be fathers and incorporate the corrections for multi-paternity and sampling error of progeny (see Appendix).

Is the reproductive success of males associated with their size?

The sizes of males were measured in six of the samples. We have used the same simple measure of reproductive success as before—the ratio of observed/expected genotype frequencies in fathers (see table 4). If both variables are ranked within each of the six experiments, none of the rank correlation coefficients is statistically significant. This is hardly surprising since there are only five genotypes available for ranking. However, we can combine the data from the six samples to yield 30 pairs of ranks. The largest genotype in each sample is ranked equally, but each has its own success ranking from 1-5. Similarly, the second largest, third largest, fourth largest, and finally, the smallest, each has its own success ranking. This allows an assessment of the general correlation between size and success in spite of the fact that there are obvious differences in the sizes of animals between samples. This analysis reveals a strong negative association between male size and reproductive success ($r_s = -0.570$, $p = 0.001$). Furthermore, there is highly significant concordance between the samples in the relationship between size and success ($w = 0.65$, $p = 0.004$). In other words, the mean reproductive success of males of a given

genotype increases as the mean size of the genotype decreases, and does so in a very consistent way. Clearly the variation in mating success between genotypes in these experiments cannot be explained in terms of the genotype-independent advantage to large males observed in the laboratory (Butlin *et al.*, 1982*a*). If the present results are accepted as representative of the real pattern of mating success in natural populations, then the previous laboratory experiments must either have missed a genotypic effect large enough to obscure the advantage to large males, or it must have artificially induced the success of large males. Note that the differences in mean size between genotypes in these samples are similar to the size differences between pairs of males in the laboratory studies. However, the size variation within each genotype is large.

Is the time of eclosion associated with male reproductive success?

There is a strong association between the time taken for seaweed flies to develop from egg to adulthood, and the chromosome I inversion (Day *et al.*, 1982). Development time is also closely associated with adult size (Butlin and Day, 1984)

Table 4 Reproductive success and mean sizes of males

Sample		Adh Genotype				
		BB	BC	BD	CD	DD
Pilot	Size	7·72	6·76	7·20	6·50	6·04
	Success	0·83	0·58	0·39·	2·05	1·82
1	Size	8·82	8·18	7·66	6·16	5·92
	Success	0	0·48	1·09	3·80	0·58
3A	Size	10·05	8·90	9·19	8·74	7·39
	Success	0·76	0·40	0·78	1·64	1·67
3B	Size	9·70	8·97	8·71	8·25	7·24
	Success	0	1·06	0·50	2·90	1·68
3C	Size	10·16	9·53	9·41	8·36	7·75
	Success	0	0·35	0·52	3·38	3·74
3D	Size	9·80	9·05	8·57	7·98	7·17
	Success	0	1·66	0·70	1·41	3·30

Reproductive success is the ratio of observed frequencies of fathers to expected frequencies (based on the observed frequencies of males available to be fathers incorporating the corrections for multi-paternity and limited sampling of progeny—see Appendix). The mean size of males are expressed in graticule units (1 unit = 0·64 mm)

such that genotypes which develop rapidly produce small adults. This could have affected our results because the males apparently available may have contained some immature individuals. In this case there could be a bias towards the later emerging genotypes (BB, BC and BD) relative to those actually capable of fertilising females. This would be aggravated by the fact that the females in a sample may have mated some time before the sample was taken. However, in natural populations, eggs are not laid synchronously so that the emergence of the different genotypes is far from being strictly sequential. Butlin (1983) found that the Adh-B frequency in male flies emerging from a sample similar to those used in experiments 1, 2 and 3, changed from 0–0·30 in the first 2 days of emergence, to 0·37 in 4 days, and very little subsequently. The females emerged throughout this period with no change in genotype frequency. The changes in expected frequencies (table 3) show the same sort of pattern in experiments 2 and 3 with only a slight increase in Adh-B and decrease in Adh-D between cages.

Males and females mate first about 24 hours after eclosion and thereafter both sexes mate repeatedly (Butlin, 1983). That several males can contribute to a female's offspring is apparent in the present results and from previous laboratory work (Butlin et al., 1982a).

Combining these observations it is clear that development rate variation is unlikely to have a

major effect on our results. Any effect it did have would be most pronounced in the early samples (2A and 3A) which should show higher mating success for rapidly developing genotypes (CD and DD) than subsequent samples. In fact there is no consistent trend in this direction (table 3).

It remains possible that rapid development or early emergence contribute in some indirect way to the mating success of the CD and DD genotypes. However, the observed variation in mating success cannot be an artefact of changing genotype frequencies over the emergence period.

Is the reproductive success of females associated with their Adh genotype and size?

Are females that laid fertile eggs a random sample of the females present? For six samples the answer is positive (see table 5). However, there is a significant departure from randomness in two samples, where there is either an excess of BB's or a deficiency of DD's among the mothers. Indeed in most samples there is a tendency for the reproductive success of BB's and BC's to be above average and for DD's to be below average.

In all four samples in which female sizes were measured there is no correlation of female size with reproductive success ($r_s = 0.014$, $p > 0.9$). There is a tendency for the larger females to be more fertile but this does not even approach statistical significance. However, when the sizes of females that laid eggs are compared (within each genotype) with those that did not, in nearly every case (19 out of 20) the fertile females were larger (data not shown). At the time of sampling it may only be the largest females that still have unlaid eggs available to be analysed in this experiment. This may be the explanation—admittedly a rather trivial one—for the apparent reproductive success of BB and BC females (the largest and youngest genotypes), and the lack of success of DD's (the smallest and oldest genotype).

In summary, we have presented evidence that under reasonably natural conditions, there are highly significant differences in the reproductive success of males with respect to their Adh genotype. There is a negative correlation between mean male size and mating success of genotypic classes. The results could be influenced by variation in emergence time. This effect is expected to be small relative to the observed variation in mating success, and there is no evidence for its occurrence in the successive samples of experiments 2 and 3.

The reproductive success of females is usually random with respect to both Adh genotype and

Table 5 *Adh* Genotype Frequencies among Females and Mothers

Sample		BB	BC	BD	CD	DD	n	χ^2	df	p
Pilot	Females	10·4	6·6	44·3	14·8	24·0	183			
	Mothers	16·1	6·5	41·9	16·1	19·4	31	0·2	3	0·98
2A	Females	26·8	9·3	45·4	3·1	15·5	97			
	Mothers	54·2	8·3	37·5	0	0	24	7·1	1	0·008
2B	Females	16·2	9·0	54·1	6·3	14·4	111			
	Mothers	20·8	11·7	46·8	7·8	13·0	77	2·7	4	0·61
2C	Females	21·3	16·4	45·9	11·5	3·3	61			
	Mothers	22·0	19·5	46·3	12·2	0	41	0·2	3	0·98
3A	Females	8·8	8·0	40·2	10·3	32·6	261			
	Mothers	13·1	6·6	45·9	13·1	21·3	61	4·8	4	0·31
3B	Females	7·8	9·0	41·3	13·8	28·1	334			
	Mothers	6·9	11·7	53·1	13·1	15·2	145	15·0	4	0·005
3C	Females	8·6	8·6	49·7	10·9	22·3	175			
	Mothers	4·1	9·6	56·2	12·3	17·8	73	3·2	4	0·52
3D	Females	3·3	6·6	54·1	14·8	21·3	61			
	Mothers	6·3	12·5	56·3	6·3	18·8	16	2·2	4	0·70

size. The departures from randomness that were observed in two samples, may well be a consequence of only the larger and younger females still having eggs available to be analysed.

DISCUSSION

In laboratory experiments large male seaweed flies father most of the progeny with inversion karyotype apparently having little influence on mating success (Butlin *et al.*, 1982*a*). Under more natural conditions there is variation between genotypes in male success correlated with the average sizes of genotypes, but small genotypes are the most successful. The reasons for this difference must lie in the design of the two experiments and need to be resolved before the influence of sexual selection on the chromosome I inversion polymorphism can be analysed.

Variation in emergence times of the genotypes from natural seaweed deposits was a potential confounding factor in the present set of experiments. We have argued that its effect on mating success was likely to be small, and in any case would have been confined to the early samples. In fact the pattern of mating success is strikingly consistent over all samples.

The conditions under which mating occurred in the two sets of experiments were very different. In the laboratory trials, individual 3-day-old virgin females were confined for 24 hours in small vials with two males. By contrast, in the more natural experiments described here, hundreds of flies with approximately equal numbers of males and females occupied large and complex habitats, and females probably remained virgin for no more than 24 hours. It is possible that the females in the laboratory experiment were exposed to many more mating attempts and, being unable to escape or hide, mated many times. This would almost certainly have been encouraged by the females' highly receptive state (being 3-day-old virgins), and could have favoured large males which are known to be able to mate more often than small males (Butlin and Day, 1985). In such a situation any female preference for particular male genotypes could have been masked, especially as there is sperm mixing (Butlin, 1983). Females in the "natural population" experiments would have had much more opportunity to exercise preferences or to avoid remating by retreating to crevices in the seaweed. In summary, we consider that the discrepant results obtained from the two experiments are a consequence of different levels of sex starvation, and habitat complexity.

Nevertheless, other factors may also be involved. The laboratory study involved only two genotypes, *BB* (chromosomally $\alpha\alpha$) and *DD* ($\beta\beta$), and the flies were from lines that had experienced some inbreeding and adaptation to laboratory culture conditions. In the population experiments all genotypes were present in natural frequencies with *BD* ($\alpha\beta$) and *DD* being the most common genotypes. This could certainly be a reason for a stronger genotypic effect observed in the latter

case. The physical conditions at the time of mating, such as light, temperature and the decomposed nature of the seaweed, were also different.

We consider the present experiments come close to revealing the pattern of mating success in natural populations. We therefore suggest that the success of large males in the laboratory trials was influenced by the unusual conditions of the experiment. It remains possible that within genotypes there is an advantage to large males in natural conditions as has been described in many insect species (e.g., Ewing, 1964; Monclus and Prevosti, 1971; Thornhill, 1976, 1983; Rutowski, 1980; Baldwin and Bryant, 1981; Boggs, 1981; Woodhead, 1981; Johnson, 1982; McLain, 1982; O'Neill and Evans, 1983; Ward, 1983).

At present we do not know the behavioural basis for variation in mating success between genotypes. We have observed no aggression between males in the vicinity of females, and no long-distance courtship behaviour. The male and female only appear to interact after mounting and before copulation, and it is not uncommon for females to dislodge males at this stage. The possibility of female choice therefore exists. Small male size could possibly be the proximate character chosen by females but many others could be involved. The chromosome I inversion includes nearly 10 per cent of the genome and may therefore influence numerous phenotypic traits. If any of these was involved in mate choice an indirect association between mating success and both genotype and size would result. It is possible that female behaviour varies with chromosome I genotype, producing departures from random mating as well as variation in mating success. This issue is considered in the accompanying paper (Day and Butlin, 1987). Mating behaviour in seaweed flies is currently under investigation.

All natural populations of seaweed flies so far studied in the British Isles are polymorphic for the chromosome I inversion and the associated *Adh* alleles (Butlin *et al.*, 1982b; Butlin, 1983). The α frequency is usually in the range 0·3–0·4 but there is evidence for a seasonal cycle with α increasing in the summer and decreasing in the winter (Butlin, 1983; Day *et al.*, in prep.). The polymorphism is maintained by heterokaryotypic advantage in viability (Butlin *et al.*, 1984) but inversion frequencies must also be influenced by development times and variation in mating success. Clearly, an improved understanding of the sexual component of selection in seaweed flies is needed before we can satisfactorily account for this polymorphism involving a substantial fraction of the complete genome.

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APPENDIX

Calculation of expected frequencies among fathers

We describe here the modifications to expectations resulting from multiple paternity and sampling error of progeny. We make the following assumptions:

(a) There are no fecundity differences between males, nor between females.

(b) The probability of a mating between any two genotypes is determined solely by the genotype frequencies in the two sexes. Thus the probability of a mating between a BB female and a BD male is the product of the frequency of BB's among females and the frequency of BD's among males.

(c) The probability of a second or subsequent mating is determined solely by the relevant genotype frequencies, and is independent of a previous mating.

(d) There are no differences in the survival of zygotes related to their Adh genotype.

(e) Broods resulting from three or more inseminations are not considered. While these assumptions may not be met precisely, they are necessary for the construction of a workable model. Small departures are unlikely to affect the conclusions.

Let p, q, r, s and t be the observed frequencies among the males available to be fathers, of Adh genotypes BB, BC, BD, CD, and DD respectively; and p, q, r, s and t be the observed frequencies among mothers; and x be the probability of a given female being inseminated by two males.

The expected frequencies among fathers ($\exp fBB$, $\exp fBC$ etc) have two components—one derived from unipaternal broods, and the other from bi-paternal broods:

$$\exp fBB = (1-x)p + xp^2$$

$$\exp fBC = (1-x)q + x(q^2 + 2pq)$$

$$\exp fBD = (1-x)r + x(r^2 + 2rp + 2pt + 2rt)$$

$$\exp fCD = (1-x)s + x(s^2 + 2st)$$

$$\exp fDD = (1-x)t + xt^2$$

and the frequency of multi-paternal broods expected to be detected is:

$$fMP = 2x(ps + qr + qs + qt + rs)$$

In order to correct these expectations for sampling error resulting from only a limited number of each brood being sampled, we have considered the Mendelian ratios in the progeny for every genotype of the mother, and every combination of two males. Assuming that 10 animals were examined from each brood, it is then easy to calculate the probability of each type of double mating being scored in any particular way. For example, if a CD female was inseminated by both a BC and a CD male, then the probability that the inferred father will be BC is 0·234. (Among the 10 progeny there must be at least one BC or BD, and at least one CC, and no DDs.) By similar reasoning the probability that the inferred father will be a BD is 0·039, and that it will be CD is also 0·039. The chances that the mating will be scored as multi-paternal is 0·656.

(In this case among the progeny there must be at least one each of *CC*, *DD* and either *BC* or *BD*.) It is further assumed that the probability of such a mating occurring is simply the product of the mother's genotype frequency and of the frequencies of the two males involved. In the example given above the probability of these three animals being involved is the product of *s*, *q*, and *s*.

The expected frequency among the fathers is then summed over all possible combinations of matings. This leads to corrected expressions for exp f*BB*, exp f*BC* etc and for f*MP*. By substituting the observed frequency of multi-paternal broods in the equation for f*MP*, an estimate of *x*, the actual frequency of double insemination, can be obtained. This can then be used to obtain values for the expected frequencies of each genotype.

The general effect of these corrections is to reduce the expectation for homozygotes, and increase them for heterozygotes. In practice, the corrections for progeny sampling error is small (approximately 1–2 per cent), but the correction for multiple insemination can be of greater consequence. An example will serve to illustrate the effect of the corrections. The following table shows the expectations of the fathers' frequencies in 3B (the largest sample) when calculated simply from the frequencies in males, and when calculated with corrections for multi-paternity and progeny sampling error.

	Adh Genotype						
	BB	*BC*	*BD*	*CD*	*DD*	χ^2_4	*p*
Inferred nos. of fathers	0	9	39	47	36		
Expected nos. of fathers (without correction)	10·1	8·8	69·4	15·7	27·1	34·9	≪0·001
Expected nos. of fathers (with correction)	7·3	8·5	77·4	16·2	21·5	38·1	≪0·001

The observed data deviate very significantly from both sets of expectations, but the comparison with the corrected expectations yields a slightly larger value of χ^2.