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RESEARCH ARTICLE

Inbreeding, inbreeding depression and extinction

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Abstract Inbreeding is unavoidable in small, isolated populations and can cause substantial fitness reductions compared to outbred populations. This loss of fitness has been predicted to elevate extinction risk giving it substantial conservation significance. Inbreeding may result in reduced fitness for two reasons: an increased expression of deleterious recessive alleles (partial dominance hypothesis) or the loss of favourable heterozygote combinations (overdominance hypothesis). Because both these sources of inbreeding depression are dependent upon dominance variance, inbreeding depression is predicted to be greater in life history traits than in morphological traits. In this study we used replicate inbred and control lines of *Drosophila simulans* to address three questions:1) is inbreeding depression greater in life history than morphological traits? 2) which of the two hypotheses is the major underlying cause of inbreeding depression? 3) does inbreeding elevate population extinction risk? We found that inbreeding depression was significantly greater in life history traits compared to morphological traits, but were unable to find unequivocal support for either the overdominance or partial dominance hypotheses as the genetic basis of inbreeding depression. As predicted, inbred lines had a significantly greater extinction risk.

Keywords *Drosophila simulans* · Dominance · Extinction risk · Purging

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Introduction

Inbreeding, the mating between two related individuals, is unavoidable in the small, fragmented or isolated populations typical of many threatened species (Frankham et al. 2002), and can lead to a significant reduction in population fitness (Keller and Waller 2002). Inbreeding depression, the decline in trait values as a result of inbreeding, has been widely reported in captive (Ralls et al. 1988), laboratory (Charlesworth and Charlesworth 1987, 1999; Falconer 1989; Roff 1997) and, increasingly, in wild populations (Crnokrak and Roff 1999; Keller and Waller 2002). The deleterious effects of inbreeding on individual fitness can be large (Roff 1997), and may be an important factor contributing to population extinction (Frankham 2005). Although empirical evidence regarding the potential involvement of inbreeding in extinction risk is scarce, increased extinction risk due to inbreeding has been demonstrated in a limited number of laboratory studies, where other factors have been controlled (Frankham 1995a; Bijlsma et al. 1999, 2000; Reed et al. 2002, 2003). Furthermore, despite the difficulty in isolating genetic effects from ecological effects in natural populations (Bijlsma et al. 2000), a direct link between inbreeding and extinction risk has been shown in the plant *Clarkia pulchella* (Newman and Pilson 1997) and in wild populations of the Glanville fritillary butterfly (*Melitaea cinixia*: (Saccheri et al. 1998). Inbreeding depression thus has potential significance for the management and conservation of endangered species (Hedrick and Kalinowski 2000), and it is important that the causes, costs and patterns of inbreeding depression are well understood.

The effect of inbreeding on trait values varies considerably between different trait types (Roff 1997: pp 321–325) and between populations (Pray and Goodnight 1995).

The effect of inbreeding on a given trait depends upon the proportion of directional dominance in that trait (Falconer 1989: pp 227; Roff 1997: pp 306; Lynch and Walsh 1998: pp 257). Because life-history traits are more closely linked to fitness they are predicted to be under strong selection (Falconer 1989: pp 225). Selection removes deleterious alleles, whilst alleles that confer a fitness advantage approach fixation. This process diminishes additive genetic variance, whilst mutations that persist are typically deleterious and recessive resulting in a relatively larger dominance variance component for traits closely related to fitness. Traits under weaker selection such as morphological characters, are expected to have relatively less dominance variance and less directional dominance (Lynch and Walsh 1998: pp 270). Hence, we expect that inbreeding depression will be more pronounced in life history traits than other characters such as general morphology. Life history traits do indeed tend to show larger dominance variance components than morphological traits (Crnokrak and Roff 1995) and should, consequently, be more susceptible to inbreeding depression.

Most reported estimates of inbreeding depression concern traits that are important fitness components: fewer have focused on inbreeding depression in morphological characters, or have directly tested the theory that inbreeding depression should be greater in life history traits compared to morphological traits. Roff (1998) tested this idea in the sand cricket *Gryllus firmus* and reported large reductions in growth rate and fecundity of inbred individuals, as well as an overall significantly higher level of inbreeding depression in life history traits than morphological traits. A meta-analysis of inbreeding depression estimates for different trait types in animals provided further support (DeRose and Roff 1999). In contrast, Ellmer and Andersson (2004) found no significant difference in the degree of inbreeding depression in traits closely related to fitness compared to morphological and phenological characters in the annual plant *Nigella degenii.* Nevertheless, they did report a trend towards greater reduction in fitness-related traits, and their sample size was small, with only two fitness-related traits assessed.

While directional dominance is a requirement for inbreeding depression to occur, the genetic basis of inbreeding depression continues to be debated. Inbreeding acts to change the frequency of genotypes in a population, increasing homozygote frequency at the expense of heterozygotes, and as a result inbreeding depression can occur via two paths. Firstly, inbreeding depression may result from the increased expression of deleterious recessive or partially recessive alleles, which are masked in heterozygotes, but are exposed as homozygosity increases (the partial dominance hypothesis). Alternatively, heterozygotes may have superior fitness to either homozygote, and inbreeding depression results from the loss of favorable heterozygote combinations (the overdominance hypothesis) (Charlesworth and Charlesworth 1987, 1999).

With continued inbreeding, the partial dominance theory predicts that natural selection will purge the deleterious recessive alleles that cause inbreeding depression, with purging being more efficient as homozygosity increases and deleterious alleles are increasingly exposed to selection. Hence, theory predicts that purging will restore the fitness of inbred populations. In contrast, the overdominance theory does not predict any purging and mean fitness will continue to decline as fewer and fewer heterozygotes are found in the population (Lande and Schemske 1985; Barrett and Charlesworth 1991; Roff 2002).

The partial dominance theory is generally considered to be the major cause of observed inbreeding depression (Charlesworth and Charlesworth 1987, 1999; Barrett and Charlesworth 1991; Dudash and Carr 1998; Roff 2002), but evidence also exists in support of the overdominance hypothesis (Karkkainen et al. 1999; Li et al. 2001), and both mechanisms may be at work concurrently (Crnokrak and Barrett 2002; Kristensen and Sorensen 2005). Since purging is only achieved when inbreeding depression is caused by deleterious recessives, a fitness rebound in inbred populations provides support for the partial dominance mechanism (Roff 2002). Purging effects have been confirmed experimentally in a number of cases (Barrett and Charlesworth 1991; Saccheri et al. 1996; Roff 2002; Swindell and Bouzat 2006b), but overall, the evidence for purging in plant and animal populations is limited and this has led to a questioning of the role of purging in restoring fitness (Ballou 1997; Byers and Waller 1999; Crnokrak and Barrett 2002; Frankham 2005).

Experimentally crossing different inbred lines offers a method of distinguishing between the two mechanisms that may cause inbreeding depression, because the two theories make different predictions about trait values of the crossbred progeny. The overdominance theory predicts that the mean trait value of crossed lines will return to the equivalent of the outbred population, as heterozygosity will be restored. In contrast, the partial dominance theory predicts that mean trait value will exceed that of the outbred population, since not only will heterozygosity be restored, but the crossbred individuals will be purged of their genetic load (Barrett and Charlesworth 1991; Roff 2002).

In this study we followed the basic experimental designs used by Roff (1998, 2002), but used *Drosophila simulans* as the model organism in order to test 3 hypotheses: 1) is inbreeding depression greater in life history than morphological traits? 2) which of the two dominance hypotheses is the major underlying cause of inbreeding depression? 3) does inbreeding elevate population extinction risk? To test hypothesis 1 mean trait values of multiple lines of inbred

individuals, subject to moderate inbreeding (expected inbreeding coefficient (F) = 0.25), were compared with outbred controls for a suite of life history and morphological traits. To differentiate between the two hypothetical causes of inbreeding depression (hypothesis 2) we maintained replicate inbred lines for seven generations of full-sibling mating, then crossed all the extant lines, and compared trait values with those of outbred controls. The maintenance of these lines also allowed us to test hypothesis 3 that inbreeding increases extinction risk, by comparing extinction of inbred and outbred lines throughout the inbreeding procedure.

Materials and methods

Foundation population

The stock population of *D. simulans* was founded from twenty isofemale lines, supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. Isolines had been cultured from individuals caught from a wild population at Tuncurry, Eastern Australia in March 2004. Isolines were mixed to produce a large outbred population and subsequently maintained in a population cage with a standing density of between 500 and 1000 flies. All experimental individuals were derived from this population after it had undergone at least 15 generations of outbreeding. Stock and experimental populations were reared throughout on Drosophila quick mix medium (Blades Biological, UK) with yeast and water at 25°C and 12/12 h light/dark cycle. Carbon dioxide or ice anaesthesia was used for handling and transferring flies.

Experiment 1: Inbreeding effects on life history and morphological traits

Replicate inbred and outbred lines were generated using a crossing design that followed Roff (1998). Eggs were collected from the stock population by placing vials of medium into the population cage and, upon eclosion to adults, these individuals formed the grandparental generation for the present experiment. Two hundred virgin flies (100 male, 100 female) were randomly taken from the grandparental generation and paired, generating 100 male–female pairs, which were placed in separate culture vials/pair. One hundred full-sibling families were obtained from these vials. Full-sib families were then randomly grouped into pairs, giving 50 groups of two full-sib families. Virgin flies from each family in each group were crossed as indicated in Fig. 1, to generate multiple inbred and outbred

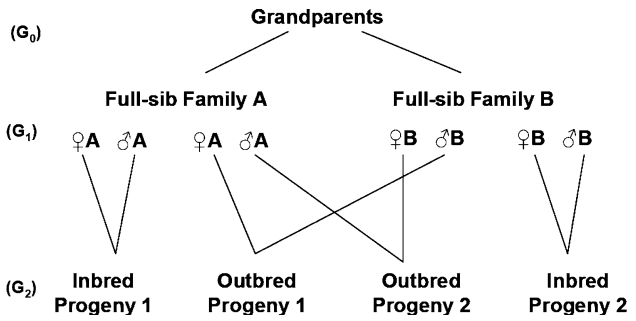

Fig. 1 Crossing design used to generate inbred and outbred progeny for a single group. Within each group, two inbred progeny families produced by brother–sister matings, and two outbred progeny families produced by reciprocal crosses between the two families. G_2 flies were the experimental individuals

lines. Within each group, two inbred lines were produced by brother–sister matings and two outbred lines were produced by reciprocal crosses of a male and female from each parental family in the group. Half of the progeny lines were therefore products of one generation of full-sib inbreeding (F = 0.25) and the other half were outbred controls. This crossing design was chosen because it should result in an equal representation of alleles (only their combinations changing), and the same proportions of dominance variance, within each group (Roff 1998).

Sample progeny were collected as virgins from each of the inbred and outbred lines, and used to measure a suite of life history and morphological traits. Due to labour constraints the experiment was carried out in three blocks of 11, 21 and 18 groups respectively. Throughout the inbreeding procedure, all flies were 3 days of age when mated.

The following traits were measured in all inbred and outbred families. The first four are classified as life history traits, the remaining four as morphological traits.

Development time

After mating of the parental generation, males were removed and females allowed to oviposit for 24 h. Adult offspring that emerged each day were removed and counted until eclosion ceased. The reciprocal of development time was calculated for the analysis of inbreeding depression, since inbreeding depression would be seen as an increase in development time and give a larger value than controls, when the opposite is required for the analysis.

Female lifetime productivity

Virgin female progeny from each line were mated once with a single outbred virgin male taken randomly from the

stock population. Both males and females were 3–4 days old when mated. Males were removed and females housed alone to oviposit, transferred to a new vial every 3 days for the first 3 weeks, then every 5 days until eclosion ceased, to avoid overcrowding of larvae. Total number of offspring that emerged from all vials per female was recorded. This measure encompasses not only female fecundity but also egg-to-adult survival of offspring. Wherever possible lifetime productivity values of two females per line were obtained.

Longevity of virgins

Number of days from emergence to death was recorded for two individuals per line. In half of the groups male individuals were used to measure longevity and in the other half of the groups, females were used. All were housed in same-sex pairs and transferred to new vials every 7 days.

Longevity of mated females

The females used to measure lifetime productivity were kept until their death so a measure of longevity of singly mated females was also obtained for two females per line.

Wing length

Length of the 1st posterior cell was measured, from the junction of the 3rd longitudinal vein with the anterior cross vein, and the border of the wing (points A–B, Fig. 2i). Both wings of each individual were measured and an average value calculated.

Wing width

Distance between the junction of the costal vein and the 1st longitudinal vein at the top right-hand corner of the costal cell, and the point where the 5th longitudinal vein meets the border of the wing was measured (points C–D, Fig. 2 ii). Both wings of each individual were measured and an average value calculated.

Hind-leg length

Length of the tibia from the joint with the femur to the joint with the metatarsus was measured (points E–F, Fig. 2iii). Both hind-legs of each individual were measured and an average value calculated.

Head width

Viewed from the front, the broad area of the face was measured between the inner borders of the great compound eyes, above the antennal foramen, using the uppermost orbital setae as a guide (points G–H, Fig. 2iv).

To measure morphological traits, adult samples were preserved by freezing at –18°C and later dissected, then photographed and measured using SPOT (Basic) for

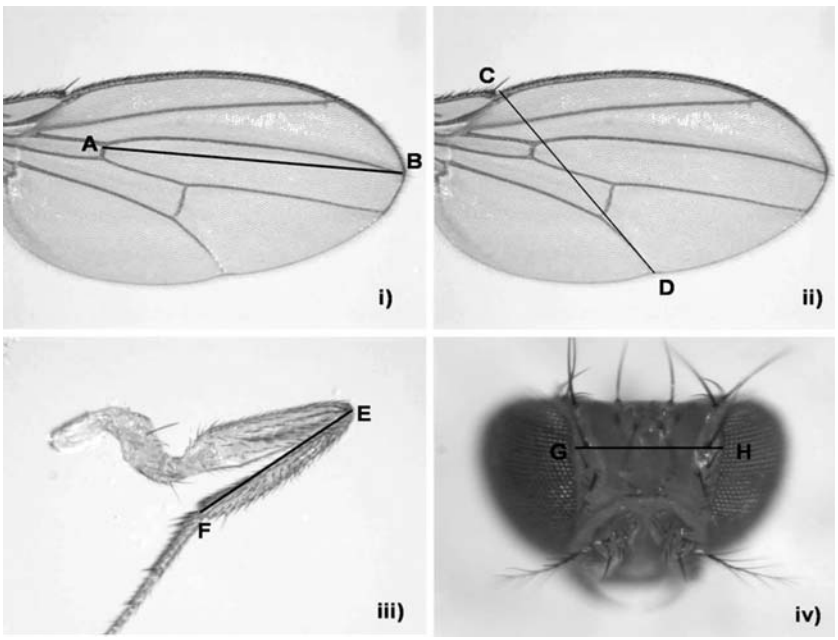

Fig. 2 Morphological measurements i) Wing length measured between points **A** and **B**; ii) Wing width measured between points **C** and **D**; iii) Hind-leg length measured between points **E** and **F**; iv) Head width measured between points **G** and **H**

Table 1 Repeatability of morphological measurements (Lessells and Boag 1987)

Variable	Repeatability	$F_{23,46}$
Wing length	0.96	596.0
Wing width	0.93	301.3
Hind-leg length	0.87	157.1
Head	0.93	330.6

windows 4.1 (Diagnostic Instruments Inc.). Because males and females of the species differ in size, only females (two per line) were used to obtain the morphological measurements. The terminology used to describe the morphological measurements follows Demerec (1994). Repeatability of each of the morphological measurements was calculated following Lessells and Boag (1987) (Table 1).

Experiment 2: Test of the overdominance or partial dominance theory and extinction rates

The design of this experiment followed Roff (2002). The same crossing design was performed as above, but 55 groups were set up, since it was anticipated that a number of the lines would become extinct as the experiment proceeded. Of the 220 possible lines, 213 were successfully established (107 inbred lines and 106 outbred lines) and these inbred and outbred lines were maintained for seven generations. Inbred lines were continued by collecting sibling offspring as virgins at each generation and randomly choosing one brother–sister pair as parents for the subsequent generation. Outbred lines were maintained by mating a single virgin female from each line with a randomly selected male from the stock population. Virgin offspring were collected from the second day of emergence throughout. Extinction of inbred and outbred lines was recorded at every generation.

At generation 7 all the remaining inbred lines (expected $F = 0.785$: see Saccheri et al. 1999) were randomly grouped into pairs and crossed reciprocally, using males and females randomly chosen from each line in the pair. Inbred and outbred lines were also continued for one additional generation as described above. For all line types females were mated once, then the males were removed and oviposition was allowed for 24 h. Progeny emerging from each vial were removed and counted daily until eclosion ceased, and mean development time for each inbred, outbred and crossbred line was recorded. Due to time constraints, no additional traits were measured.

Statistical analysis

Inbreeding depression was estimated for each trait by calculating the coefficient of inbreeding depression (σ):

$$\delta = 1 - X_I/X_o \quad (1)$$

where X_I is the mean inbred trait value and X_o the mean outbred trait value. This was done for each individual line and each group. An overall mean outbred value was calculated for each trait using data from all outbred progeny lines (X_o), or, when block had a significant effect on the trait values, a mean outbred value for each block was used. The mean inbred value for each inbred line and each group replaced X_I, resulting in replicate estimates of the inbreeding coefficient for each trait, from which an overall mean estimate was obtained. All subsequent analysis was performed using SPSS 11.5 for windows.

Results

Experiment 1: Inbreeding effects on life history and morphological traits

Of the 200 possible lines from 50 groups, 166 were successfully established and 34 failed to produce sufficient progeny. In total 48 groups that contained at least one inbred and one outbred line were obtained, of which 28 groups were completely balanced (see Fig. 1). A population mean value of the inbreeding depression coefficient (σ) was estimated for each trait in two ways; firstly by generating a σ value for each inbred line, by comparing the inbred trait value for that line with the outbred population mean, then calculating a population mean σ for each trait using the σ values for all lines (subsequently referred to in the text as analysis using 'line' data). Secondly, a σ value was estimated for each group by comparing mean inbred values from the four lines within the group to the population outbred mean, then calculating an average for σ each trait from all the group values (subsequently referred to in the text as analysis using 'group' data). These analyses only used the fully-balanced groups.

Inbreeding depression coefficient (σ) values varied substantially both among traits and among inbred lines within traits (Table 2). When calculated using the line data, population mean σ estimates (×100 for ease of comparison) for traits ranged from 0.5 for wing width, to 11.94 for longevity (virgins), and from 0.43 for hind-leg length to 11.65 for longevity (virgins) when calculated using the group data. All life history traits showed greater population mean inbreeding depression estimates than morphological traits (Fig. 3).

Table 2 Inbreeding depression estimates calculated using line data and group data

Trait[a]	$\delta \times 100$		Sample size		P value[b]
	Mean ± SE	Range	Inbred	Outbred	
Line data					
Development time	1.97 ± 0.64	–8.12–26.35	82	84	0.021*
Longevity (virgins)	11.94 ± 3.79	–56.84–84.45	81	82	0.029*
Longevity (mated females)	10.49 ± 2.33	–32.97–56.09	82	84	0.001*
Lifetime productivity	10.62 ± 4.21	–94.51–100.00	81	84	0.087
Wing length	1.00 ± 0.32	–5.27–8.40	82	83	0.025*
Wing width	0.51 ± 0.30	–5.60–8.40	82	83	0.324
Hind-leg length	0.60 ± 0.32	–5.49–8.97	82	83	0.211
Head width	1.27 ± 0.44	–5.49–16.06	81	83	0.150
Group data					
Development time	2.24 ± 0.71	–7.48–14.71	48	48	0.019*
Longevity (virgins)	11.65 ± 4.27	–30.93–84.44	47	47	0.006*
Longevity (mated females)	10.01 ± 2.83	–27.95–56.10	48	48	0.006*
Lifetime productivity	9.61 ± 5.67	–94.51–100.00	48	48	0.123
Wing length	0.98 ± 0.34	–3.88–7.39	48	48	0.042*
Wing width	0.50 ± 0.31	–3.88–5.58	48	48	0.231
Hind-leg length	0.43 ± 0.36	–5.53–6.73	48	48	0.436
Head width	1.21 ± 0.48	–5.51–13.03	47	47	0.161

* Significant at $P < 0.05$ level

[a] Bold type represents life history traits, normal type represents morphological traits

[b] P values were calculated using t-tests, Mann Whitney U-tests, or one-way ANOVA for line data, and Paired t-tests or Wilcoxon Signed Ranks-tests for group data

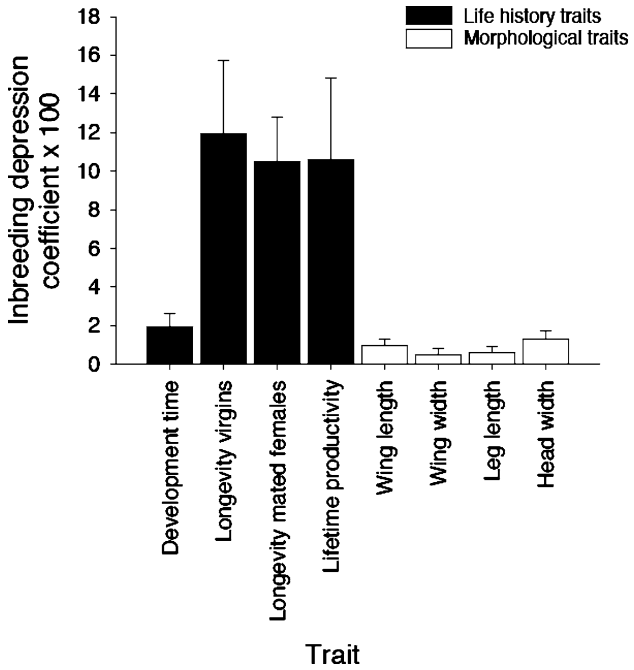

Fig. 3 Population mean inbreeding depression coefficient estimates (×100, ± SE) for life history traits and morphological traits based on line data

When analyses were performed on the line data, mean development time and lifetime productivity were significantly affected by block (Kruskall-Wallis: $\chi^2 = 61.92$, df = 2, $P < 0.001$; one-way ANOVA: $F_{2,164} = 29.02$, $P < 0.001$ respectively), and were subsequently analyzed controlling for block effects. Inbreeding significantly reduced trait values of mean development time, longevity of both mated and unmated flies, and of wing length, but not of lifetime productivity or the remaining morphological traits (Table 2). Paired t-tests or Wilcoxon Signed Ranks tests were performed on the group data, and produced very similar results (Table 2).

To examine whether life history traits show higher levels of inbreeding depression than morphological traits, inbreeding depression estimates were grouped by trait type and the two groups ('Life history traits' and 'Morphological traits') compared. We calculated an overall life history trait σ value, using the mean value of the four life history traits, for each inbred line and for each group. The same procedure was repeated for the morphological traits. Overall life history trait σ values were compared with overall morphological trait σ values for both the line data and the group data using paired analysis. Life history traits

Table 3 A comparison of overall inbreeding depression estimates for life history traits and morphological traits

	$\delta \times 100$		$\delta \times 100$		P value[a]
	Life history trait		Morphological trait		
	Mean ± SE	n	Mean ± SE	n	
Line data	8.78 ± 1.64	83	0.86 ± 0.29	82	<0.001*
Group data	8.38 ± 2.10	48	0.75 ± 0.30	48	0.001*

[a] P values were calculated using either Paired t tests or Wilcoxon Signed Ranks test

* Significant at $P < 0.01$ level

showed significantly higher inbreeding depression than morphological traits in both comparisons (Table 3).

Comparing the two trait types using population mean σ estimates for each trait in the analysis, (giving four samples in each group) also indicated significantly greater inbreeding depression in life history traits than morphological traits based both on the line data (Mann Whitney U-test $U_1 = 4$, $U_2 = 4$, $z = -2.309$, $P = 0.021$), and on the group data (Mann Whitney U-test $U_1 = 4$, $U_2 = 4$, $z = -2.309$, $P = 0.021$).

Experiment 2: Test of the overdominance or partial dominance theory and extinction rates

As the experiment proceeded, both inbred and outbred lines suffered some extinction at every generation. Extinction of lines occurred due to failure of parents to produce offspring, insufficient emergence of offspring, or emergence of offspring of only one sex. By generation eight, 73 out of 107 (68%) inbred lines and 46 out of 106 (43%) outbred lines had become extinct. Inbred lines were significantly more likely to go extinct after 8 generations of full-sib mating compared to outbred lines (Fisher's exact test: $P = 0.026$, Fig. 4).

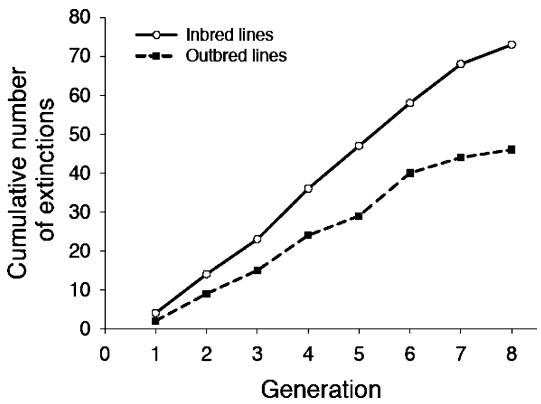

Fig. 4 Cumulative numbers of extinct lines at each generation, from 107 original inbred lines and 106 original outbred lines, during eight generations of full-sibling mating

At generation 7 the 38 extant inbred lines were crossed reciprocally, giving rise to 35 crossbred lines (3 failed to produce offspring); trait values of reciprocal crosses were combined for analysis giving a sample size of 19 crossbred lines. Data were analysed using two ANCOVA's as it was possible that the number of progeny emerging could influence mean development time and vice versa (although we got identical results with MANOVA). We first compared number of progeny emerging as a function of treatment (inbred lines, crossed-inbred lines and outbred lines) while controlling for mean development time. We found that line-type had a significant effect on progeny number ($F_{2,109} = 25.1$; $P = 0.0001$), and post-hoc tests indicated this was due to the outbred lines ($n = 60$) producing more offspring than either the inbred ($n = 34$) or inbred crossed lines ($n = 19$) which did not differ (mean offspring number ± se: outbred 72.24 ± 2.4; inbred 43 ± 3.7; crossed-inbred 45 ± 5.0. Fisher's PLSD inbred v. crossed-inbred $P = 0.69$; inbred v. outbred $P < 0.001$; outbred v. crossed-inbred $P < 0.001$). Development time was not significantly associated with any variation in offspring production ($F_{1,109} = 0.49$; $P = 0.48$). Similar analysis of the development time data revealed no significant effects (Line type $F_{2,109} = 1.47$; $P = 0.23$; Offspring number $F_{1,109} = 0.49$; $P = 0.48$).

Discussion

Effect of inbreeding on life history compared to morphological traits

The results of this study support the observation made by Falconer (1989: pp 225) that life history traits show more inbreeding depression than morphological traits. When analyzed using either the line data or the group data, all four life history traits that were measured showed higher inbreeding depression than morphological traits. When traits were grouped by type, life history traits showed overall significantly higher inbreeding depression than morphological traits. These results suggest higher directional dominance for life history traits than morphological

traits as predicted by theory (Falconer 1989: pp 225; Roff 1997: pp 321).

Mean inbreeding depression coefficient estimates (×100) for life history traits ranged from 1.97 for mean development time to 11.94 for longevity (virgins) (based on analysis using all lines), showing that some life history traits are more severely effected by inbreeding depression than others and suggesting variation in the ratio of directional dominance across traits. Our inbreeding depression values for life-history traits are close to those reported for *D. simulans* in other studies (e.g., 8% for viability: Kosuda 1980), and to some estimates for other *Drosophila* (e.g., 18% for longevity (Hughes 1995) and 18% for fertility (Tantawy and Reeve 1956) both in *D. melanogaster*). However, the overall magnitude of inbreeding depression for life-history traits in laboratory populations of *Drosophila* tend to be higher than what we report here (see Table 10.2 Lynch and Walsh 1998), even if we exclude our lowest estimate, that for development time. The low inbreeding depression for mean development time (1.97) we report suggests little directional dominance for this trait. This parallels the findings of Roff (1998) who reported low inbreeding depression (2.0) for mean development time (hatching to adult) in *G. firmus*. In comparison, longevity (virgins and mated flies) and lifetime productivity show relatively high levels of inbreeding depression (11.94, 10.49 and 10.62 respectively), indicating more directional dominance for these traits, and suggesting that they may be under stronger selection in *D. simulans* than development time. Morphological traits all showed low levels of inbreeding depression, as has been theoretically predicted for such characters indicating that they may not be under especially strong selection (Roff 1997: pp 321; Lynch and Walsh 1998: pp 270). However, some inbreeding depression was observed, which indicates morphological traits are not without directional dominance. Our estimates of inbreeding depression in morphological traits are broadly similar to those of other *Drosophila* (Lynch and Walsh 1998: pp 271). For example, inbreeding depression in wing length for *D. melanogaster* has been reported to be between 1 and 3% (Tantaway 1957; Tantawy and Reeve 1956), with the lower estimate indistinguishable from ours (Table 2). Radwan and Drewniak (2001) also report inbreeding depression of about 3% for another size measure (thorax length), which again is close to the measures we report.

Analysis of the difference in mean trait values between inbred and outbred families supported the above results with two exceptions. Firstly, no significant difference in lifetime productivity between inbred and outbred groups was seen, despite the relatively large mean inbreeding depression coefficient of 10.62. This result is due to the large variation in productivity values of inbred (range: 0–391 offspring) and outbred (range: 5–424 offspring) families. Additionally, mean wing length was significantly reduced in the inbred group, whereas the remaining morphological traits showed no significant difference in mean trait value between inbred and outbred groups. Wing length has been shown to have high levels of directional dominance in *D. melanogaster* (Gilchrist and Partridge 2001) suggesting that the trait is under strong selection and may be more closely associated with fitness than other morphological traits.

Large variation among inbred lines for both mean trait values and inbreeding depression estimates was observed in this study (see Table 2). This fits with other laboratory studies that have reported large variation in the response of replicate lines to inbreeding despite a constant inbreeding level (Fowler and Whitlock 1999; Reed et al. 2002; Kristensen et al. 2003), and an increase in phenotypic variation relative to outbred populations following inbreeding (Whitlock and Fowler 1996). In the present experiment some inbred lines showed severe inbreeding depression whilst others performed as well as or better than outbred control lines, suggesting that some populations can retain high fitness following population bottlenecks, whilst others cannot (Fowler and Whitlock 1999; Reed et al. 2002). In this study, differing numbers of deleterious mutations carried by the individuals that founded each line may have contributed to the observed variation in inbreeding depression among lines (Whitlock and Fowler 1996).

Test of the genetic basis of inbreeding depression and extinction risk

The primary aim of this experiment was to distinguish between the two mechanisms that might cause inbreeding depression by comparing traits in lines with different histories of inbreeding and different heterozygosity levels. Mean development time and productivity in the 24 h after laying were the characters assayed. While we did not expect any significant levels of purging to occur during our inbreeding procedure (the effective population size of the inbred lines was so small that only mutation with effectively lethal effects would be purged), predictions could nevertheless be made about the trait values expected under each mechanism. If the overdominance mechanism was responsible for inbreeding depression, then the crossed lines should be similar to the outbred lines. On the other hand, if partial-dominance was responsible for inbreeding depression, then we should have seen a small increase in trait values of the crossed-inbred lines relative to the inbred lines, because some of the deleterious recessives should be masked by crossing.

What we found was that the 24-h productivity of the outbred lines was far higher than either the inbred or crossed-inbred lines, which did not differ, and there was no significant difference in mean development time between the crossed lines, the outbred lines or the inbred lines. There are several possible explanations for these results. Firstly, there may be little directional dominance, and consequently little inbreeding depression for development time in *D. simulans*. Of the four life history traits measured in experiment one, development time showed the least inbreeding depression. Similarly, low inbreeding depression for development time was reported in *G. firmus* (Roff 1998). It therefore appears that development time is a poor character in which to examine inbreeding, at least in *D. simulans* and *G. firmus*. However, productivity showed substantial inbreeding depression, and while there was a very small increase in the average number of offspring produced by the crossed lines in comparison to the inbred ones, this was far from statistically significant. Nevertheless, the results are arguably supportive of partial dominance. With our experimental design we expect no purging (see above), and hence only a small increase in productivity, which is what we found, and perhaps our failure to find an effect here may be a sample size problem. Additionally, the large variation in this trait seen in all lines means detecting significant differences would be difficult, and we note that the reduction in life-time productivity with one generation of inbreeding (Experiment 1) was not statistically significant in spite of its large magnitude for this very reason: we also note here that the lack of difference between crossed and inbred lines is not due to them being assayed at different times or on different batches of food. The large variation in productivity we record here is not unusual and we find similar variation in other experiments. Perhaps the addition of more stressful rearing conditions would facilitate the detection of differences in productivity (Hoffman and Parsons 1991)? This is something we are pursuing. We also note that if both mechanisms contributed to inbreeding depression in productivity, then we should also have seen a difference between inbred and crossed lines, which we did not.

Our finding of higher extinction of inbred lines during the experiment is also arguably indicative of expression of deleterious recessive alleles; the overdominance mechanism predicts a decline in fitness but not necessarily increased extinction (Radwan 2003). The rational behind this seems to be that although under the overdominance hypothesis inbreeding results in the loss of the highest fitness class, remaining individuals do not necessarily become worse over time and their populations therefore need not have higher extinction risk. Additionally, with overdominance we would also expect to see continued decline in the inbred lines relative to the outbred lines with continued inbreeding, rather than convergence of trait values in inbred and outbred lines (Roff 1997), although the convergence we see is in a trait with little apparent directional dominance. Again however, if overdominance was responsible for inbreeding depression we should see greater productivity in the crossed lines, which we did not. Consequently it is not possible to draw any firm conclusions regarding the underlying cause of inbreeding depression in *D. simulans*, but we think the evidence weakly supportive of the partial dominance idea. Generally, evidence has been generated both in support of the role of partial dominance and for its absence. Recently, for example, (Swindell and Bouzat 2006a) reported that purging decreased inbreeding depression by 40% in *D. melanogaster*, whilst, on the other hand, Radwan (2003) found no evidence of purging in inbred lines of the bulb mite (*Rhizoglyhus robini*). At present the majority of experimental evidence has shown only limited effects of purging on fitness restoration of inbred populations (reviewed in Frankham 2005). Furthermore, the efficiency of purging may depend on the nature of genetic variance, with lethals being quickly removed, but mildly deleterious mutations persisting and even becoming fixed in the population (Hedrick 1994; Keller and Waller 2002).

Our study also reveals significantly higher extinction risk of inbred lines compared to outbred lines, supporting the notion that inbreeding increases the extinction risk of small, inbred populations. These results are in agreement with a growing number of laboratory studies that provide direct experimental evidence that inbreeding elevates extinction risk (Frankham 1995b; Bijlsma et al. 1999, 2000; Reed et al. 2002, 2003). These investigations have used not only full-sib inbreeding, but have also found increased extinction risk with lower rates of inbreeding (Frankham 1995b; Reed et al. 2003), and in populations after purging had occurred (Bijlsma et al. 2000). Overall this body of work suggests that inbreeding can increase extinction risk both through selection acting on deleterious recessive alleles via purging, or by decreasing the mean fitness and genetic diversity of the inbred population (Hedrick 1994). Furthermore, inbreeding can act synergistically with environmental stress to increase extinction probability (Bijlsma et al. 1999, 2000) and, any attempt to deliberately inbreed populations to purge them of their genetic load through close inbreeding (Templeton and Read 1984) may in fact increase the probability of extinction (Hedrick 1994).

In summary, our results provide evidence in support of theory suggesting life history traits have greater directional dominance and hence should show greater inbreeding depression than morphological traits. However, this study also indicates that morphological traits are not without directional dominance and can display low levels of

inbreeding depression, which can in turn contribute to the loss of fitness of inbred individuals. We were unable to find unequivocal support for either hypothesis regarding the underlying genetic mechanism of inbreeding depression. Finally, our study lends further support to the notion that inbreeding elevates extinction risk, and highlights the need for greater understanding of the genetic factors influencing extinction probability.

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