Biological Journal of the Linnean Society, 2009, **98**, 501–510. With 3 figures

No evidence for optimal fitness at intermediate levels of inbreeding in *Drosophila melanogaster*

STEPHEN P. ROBINSON*, W. JASON KENNINGTON and LEIGH W. SIMMONS

Centre for Evolutionary Biology, School of Animal Biology (M092), The University of Western Australia, Crawley, WA 6009, Australia

Received 10 March 2009; accepted for publication 5 May 2009

Optimal outbreeding theory predicts fitness benefits to intermediate levels of inbreeding. In the present study, we test for linear (consistent with inbreeding depression) and nonlinear (consistent with optimal outbreeding) effects of inbreeding on reproductive fitness in male and female *Drosophila melanogaster*. We found linear declines in fitness associated with increased inbreeding for egg-to-adult viability, but not the number of eggs laid or sperm competitive ability. Egg-to-adult viability was also lower in the progeny of inbred males and females mated to unrelated individuals. However, there was no evidence for optimal fitness at intermediate levels of inbreeding for any trait. The present study highlights the importance of considering biologically realistic levels of inbreeding and cross-generational effects when investigating the costs and benefits of mating with relatives.

ADDITIONAL KEYWORDS: genetic relatedness – inbreeding depression – inclusive fitness – outbreeding depression – reproductive fitness – sperm competition.

INTRODUCTION

It is well established that matings between genetically similar individuals, such as siblings, may result in offspring with reduced fitness. The reduction in fitness after inbreeding (inbreeding depression) is well documented in plants and animals for a wide number of traits (Charlesworth & Charlesworth, 1999; Keller & Waller, 2002; Carr & Dudash, 2003), and is usually attributed to either an increase in the number of deleterious recessive alleles present as homozygotes (dominance), or the loss of heterozygosity where heterozygotes are fitter than either homozygote (overdominance) (Greaves *et al.*, 1977; Borrell *et al.*, 2004).

It has also been documented that crosses between distantly related individuals may also suffer from reduced fitness, an effect known as outbreeding depression. The most extreme example of outbreeding depression is the often zero fitness observed from crosses between species. However, there are also many instances where crosses between isolated populations of the same species produce offspring of suboptimal fitness (Templeton, 1986; Alipaz, Karr & Wu, 2005). Outbreeding depression is usually attributed to the break up of locally adapted or coadapted gene complexes, so it is important to note that depression may not be exhibited until recombination begins to break apart gene complexes in the F2 or higher-order hybrid generations (Lynch & Walsh, 1998). As well as inbreeding and outbreeding depression, the relatedness of the mates that an individual chooses may also have other implications for their fitness. For example, inclusive fitness theory predicts that an individual who mates with a close relative helps that relative to spread genes identical to it by descent, thereby receiving a benefit. In a recent study, Kokko & Ots (2006) revive this the idea of inclusive fitness, suggesting that it may make up for part of the costs of inbreeding depression, such that, in many cases, inbreeding tolerance should be expected even when the costs of inbreeding depression are severe. Because of these costs and benefits to both inbreeding and outbreeding (Partridge, 1983), Bateson (1983) proposed that mating with an individual of intermediate relatedness should offer the best fitness, resulting in a pattern of optimal outbreeding.

*Corresponding author.
E-mail: stephen.robinson@graduate.uwa.edu.au

Consistent with a system of optimal outbreeding, great frigate birds, *Fregata minor*, have been found to choose a mate that is more genetically similar to themselves than would be expected under random mating (Cohen & Derhorn, 2004). Some of the best evidence for optimal outbreeding comes from studies that use a specifically bred pedigree to accurately calculate the inbreeding coefficient (Bateson, 1980). From mate choice experiments on laboratory mice, Barnard & Fitzsimons (1988) demonstrated a preference for mating with an intermediate relative, and went on to show that crosses between intermediate relatives were associated with higher reproductive output (Barnard & Fitzsimons, 1989), although no effect of mate relatedness was found for fitness variables such as pup mortality, weight, or growth rate. Similarly, female white-footed mice, *Peromyscus leucopus*, bred from wild caught animals, preferred first cousins to either unrelated males or full siblings (Keane, 1990). Litter size and weight at first weaning indicated that crosses between first cousins performed better than those between either unrelated mates or full or half siblings. Interestingly, a recent study found that, in humans of the Icelandic population born between 1800 and 1965, there is a significant positive association between kinship and fertility, with the greatest reproductive success observed for couples related at the level of third and fourth cousins (Helgason *et al.*, 2008).

Studies of inbreeding depression typically contrast fitness parameters associated with extreme inbreeding (i.e. sib matings) and outbreeding (i.e. matings between unrelated individuals). Moreover, they often focus on parameters of female fecundity, whereas measures of inbreeding depression on male fertility are rare (Hughes, 1997; Konior, Keller & Radwan, 2005). In the present study, we investigate the direct effects on fitness of continuous variation in the levels of inbreeding on male and female reproductive fitness on a recently established laboratory population of *Drosophila melanogaster*. Although indirect effects on fitness (such as inclusive fitness) are beyond the scope of the present study, it should be noted that they may play an important influence in a system of optimal outbreeding. We test for both a linear (consistent with inbreeding depression) and a nonlinear (consistent with optimal outbreeding) relationship between the extent of inbreeding and fitness.

MATERIAL AND METHODS

Fly stocks

The *Drosophila* stock used in our experiments was established from 50 wild caught females collected from a single site in Margaret River (located on the south-west coast of Western Australia, 33.95 °S, 115.07 °E).

Competitor males for sperm competition assays were brown eyed (*bwD*) dominant marker flies. The marker flies used were a replica of an LH_M population carrying a brown eye colour (*bw*) dominant marker that has been introgressed through 13 backcross generations. The LH_M population is a large (adults, $N > 1700$ per generation) outbred population that was founded in 1991 from central California. This stock was kindly made available by Professor Bill Rice, and details as to how the line was generated are provided elsewhere (Byrne & Rice, 2005). All fly stocks were maintained on standard agar-maize-yeast medium at 27 °C under a 12 : 12 h light/dark cycle in 9-Litre containers.

Generation of experimental flies

Pedigree lines were generated 6–9 weeks (four to seven generations) after the stock population was established (i.e. 8–11 weeks after the flies were originally collected). A crossing design similar to that used by Mack, Hammock & Promislow (2002), and expanded to include second cousins, was used to generate simultaneously individuals of known inbreeding coefficients: full siblings ($f = 1/4$), half siblings ($f = 1/8$), first cousins ($f = 1/16$), second cousins ($f = 1/64$), and unrelated individuals ($f \approx 0$) (Fig. 1). Flies were paired in individual 50-mL vials on 10-mL agar-maize-yeast medium, which had been seeded with a scoop of approximately 5 mg of live yeast. To ensure larval density remained low, adults were transferred to a second laying vial after 48 h, and then discarded

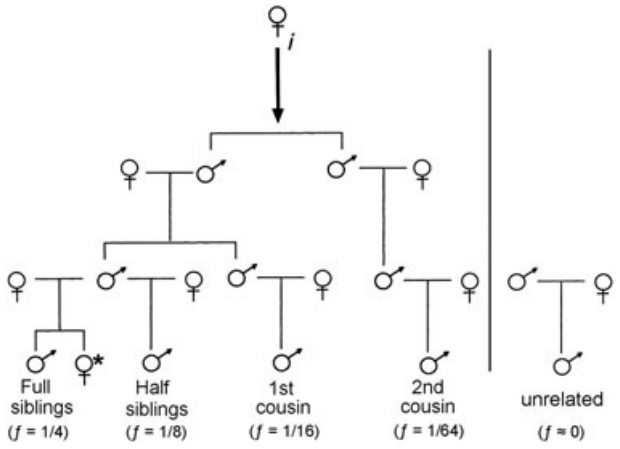

Figure 1. Crossing design used to generate the parental generation modified from Mack *et al.* (2002). The female marked with an asterisk (*) denotes the parent female line. Male labels indicate the level of relatedness to this female and inbreeding coefficient (f) of the offspring from a cross with this female.

after another 48 h. Males from these two vials were collected after 14 days using cold anaesthesia and paired with virgin females from the stock population to establish the next set of crosses. All virgins used throughout the experiment were collected under cold anaesthesia and sorted into single sex vials within 7 h of emerging, before they reached sexual maturity (Goldstein & Fyrberg, 1994). All other transfers where flies had to be sorted or separated were carried out under brief (less than 60 s) CO_2 exposure. To ensure the pedigree was complete even if some crosses produced no offspring, four replicates of each cross were established and one of these was selected at random to provide offspring for the next step in the pedigree.

After three generations, the pedigree (Fig. 1) produced parent females from a single family, which could then be crossed to males of the desired levels of relatedness (full sibs, half sibs, first cousins, second cousins, unrelated). It should be noted that, because male offspring were used to establish each subsequent generation in the pedigree (mated to either a related or an unrelated female), our crossing design did not produce any inbreeding on the X chromosome.

Where there were sufficient females, two replicates of each cross were established. The offspring from these crosses possessed the desired levels of inbreeding to be assayed for fitness, and are referred to as the F1 generation. Once each cross had been established, the females were allowed to lay for 24 h on each of the first two laying vials. These experimental parents were then moved to a third vial to assess the egg-to-adult viability of their F1 offspring. The entire procedure was replicated 24 times, with each replicate forming a single pedigree or block for analysis. For 12 of these blocks, offspring from the first two laying vials were collected as virgins and assayed for male fitness (sperm competitive ability), female fitness (egg laying), and for the egg-to-adult viability of the offspring that they produce (F2 generation).

To avoid any confounding effects of larval density, all flies derived from the mass populations, including those used to establish pedigree lines, and the unrelated individuals crossed into the pedigree, were picked as larvae from standard grape juice agar laying medium, which had been placed directly into the population cages. These flies were reared at a standard density of 50 larvae per vial.

Fitness assays

Experimental flies were maintained on vials of standard agar-maize-yeast medium at 27 °C under a 12 : 12 h light/dark cycle. Egg-to-adult viability has been considered in some studies as being fully dependent on the maternal genotype (Lopez-Fanjul & Villaverdea, 1989; Garcia, Lopez-Fanjul & Garcia-Dorado, 1994) because this may account for up to 74% of the variation (Chapco, 1979). However, Rodriguez-Ramilo *et al.* (2004) found that egg-to-adult viability had maternal effect and offspring genotype components of equal size. For this reason, egg-to-adult viability was measured for both the F1 offspring, and their offspring (F2). Because the F1 generation was mated to unrelated individuals, their offspring (F2) are outbred, and therefore any effect of inbreeding on egg-to-adult viability of the F2 generation must be the result of maternal or paternal environmental effects of the F1 generation.

Egg-to-adult viability of offspring produced by parental crosses

To measure egg-to-adult viability of offspring from the parental crosses, a focal female (marked ♀* in Fig. 1) and male of the desired degree of relatedness were paired on a nonyeasted vial and allowed to lay for 24 h. The eggs laid in this period were counted and the number of adults that had emerged after 14 days were used to calculate egg-to-adult viability. All parental females were 5–7 days old, and had been with their mate for 48 h on yeasted vials to ensure that they were mated and ready to oviposit before the assay commenced.

Sperm defense of F1 males

P1 is the proportion of a doubly mated female's total offspring sired by the first male to mate (Boorman & Parker, 1976), and is a measure of success in defensive sperm competition. To assess P1 for F1 males, five virgin 3–5-day-old males were paired with five virgin 3–5-day-old females and allowed to mate for 2 h. Multiple mating during this period was unlikely because very few females remate within 24 h of mating (Kalb, DiBenedetto & Wolfner, 1993; Chapman & Davies, 2004). The males were then collected, and the females transferred to individual nonyeasted laying vials to assess offspring viability. If the female did not produce any offspring from this mating in the subsequent 48 h, she was assumed not to have mated and the replicate was dropped from the analysis. However, most females (97%) were found to have mated. A competitor brown eye dominant (*bwD*) male was added to each vial 48 h after the initial mating and left for a period of 24 h to allow mating. Again, females are only likely to have mated once during this 24-h period. The females were then transferred to a final yeasted laying vial, the offspring from which were counted and scored for eye colour to assign paternity. Because of a strong last male precedence in this species (Clark *et al.*, 1995), it was likely that cases where the second male failed to sire any offspring were the result of the female not mating

with the second male. This was not common (only 25 out of 376 cases) and, when it occurred, the data were excluded from analysis so that only the effects of actual sperm competition were considered, rather than the effects of remating.

Sperm offense of F1 males

P2 is the proportion of a doubly mated female's total offspring sired by the second male to mate (Boorman & Parker, 1976), and is a measure of success in offensive sperm competition. An assay of P2 for F1 males was conducted in the same manner as the P1 assay, except that this time females were mated first to a *bwD* competitor male before being mated to the F1 male. Mating times and transfers were performed in the same as the P1 assay, with an initial period of 2 h for the first mating, a 48-h break to allow for females to become receptive again, and a 24-h period for the second mating. Because no viability assay was carried out, females were transferred directly to the yeasted second mating vial rather than spending 24 h on a nonyeasted viability vial.

Egg-to-adult viability of offspring produced by F1 males

Because the number of offspring sired by each male was measured as emerging adults, egg-to-adult viability of these offspring may have an influence on the measured P1 and P2. Gilchrist & Partridge (1999) suggest that reduced offspring viability could account for the variation in sperm competitive ability recorded in many studies. To correct for differences between the groups, the egg-to-adult viability of the offspring sired by males of each group was measured during the P1 assay. This assay was conducted in the same manner as that for the parental crosses (see parental cross fitness assays) except that females were not mated until 2 h before transfer to the viability vial, and did not have the male present during the egg laying period. Egg-to-adult viability was used to correct both P1 and P2.

Egg-to-adult viability of offspring produced by F1 females

The egg-to-adult viability of offspring produced by F1 females was assayed in the same manner as the parental crosses. F1 females and two unrelated males were allowed to lay eggs for 24 h on a nonyeasted laying vial. The number of eggs laid in this period was counted and the number of adults that had emerged 14 days after laying commenced was used to calculate egg-to-adult viability. All F1 females were 5–7 days old, and had been with their mate for 48 h on yeasted vials to ensure they were mated and ready to oviposit before the assay commenced.

Statistical analysis

Prior to analysis, all replicates within each block/cross combination were pooled by summing the number of eggs or adult flies (marker and wild type flies were not combined). We used generalized linear models (GLM) to analyse the effects of inbreeding (or relatedness between mates in the parental generation) on each of the fitness components measured. The models included the effect of block, inbreeding coefficient, and their interaction as explanatory variables. Because data for all traits were overdispersed (i.e. ratios of the residual deviance to residual degrees of freedom were consistently greater than one), GLM models were fitted with a quasibinomial error distribution for the proportional data, and a quasipoisson error distribution for the count data (Crawley, 2002). Explanatory variables were analysed sequentially in the order: block, inbreeding coefficient, and block-by-inbreeding coefficient interaction. Nonlinear relationships were tested by adding a quadratic term (inbreeding coefficient)^2 to each model, and testing for an improvement of fit using a chi-square test. Effect sizes were calculated for each term in the GLM models using the partial Eta squared statistic (η_p^2). Partial Eta squared is the proportion of the effect and error variance that is attributable to the effect. The estimates reported were calculated using the equation: $\eta_p^2 = SS_{between}/(SS_{between} + SS_{error})$ (Levine & Hullett, 2002). To test the robustness of our results, we also analysed the data using analysis of variance on arcsine square root-transformed data (proportional data only), and carried out permutation tests. Permutation tests were performed by shuffling trait values randomly with respect to the block and inbreeding coefficient labels, and re-calculating *F*-values. A *P*-value was obtained for each term in the model by comparing the actual value to a distribution of values generated from 1000 randomized data sets. All analyses were conducted using the R package (http://cran.r-project.org/).

RESULTS

Parental fitness

There was no significant effect of the level of relatedness on the number of eggs laid by females of the parental crosses (Table 1). However, egg-to-adult viability showed a significant linear decrease with increasing relatedness among the parents (Fig. 2; Table 1), as would be expected under classic inbreeding depression. The addition of a quadratic term did not significantly improve the fit of either GLM model

Table 1. The effect of inbreeding on reproductive fitness traits in the parental generation

Trait	Source	d.f.	Deviance	F	P			η_p^2
					Model 1	Model 2	Randomization	
Eggs laid	Block	23	634.6	2.20	**0.007**	**0.001**	**0.001**	0.442
	Relatedness	1	19.4	1.55	0.218	0.166	0.167	0.024
	Block × Relatedness	23	426.2	1.48	0.114	**0.032**	**0.025**	0.347
	Total	109	1882.5					
Egg-to-adult viability	Block	23	487.6	2.27	**0.006**	**0.005**	**0.016**	0.446
	Relatedness	1	40.5	4.38	**0.042**	**0.027**	**0.029**	0.063
	Block × Relatedness	23	156.0	0.73	0.799	0.805	0.815	0.205
	Total	109	1290.1					

The results were analysed with two models. Model 1 is a generalized linear model with a quasibinomial (proportional data) or quasipoisson (count data) error using sequential deletion for statistical inference. Model 2 presents probability values from a mixed model analysis of variance. Model 2 was also tested with a permutation test (1000 replicates). Significant terms are highlighted in bold.

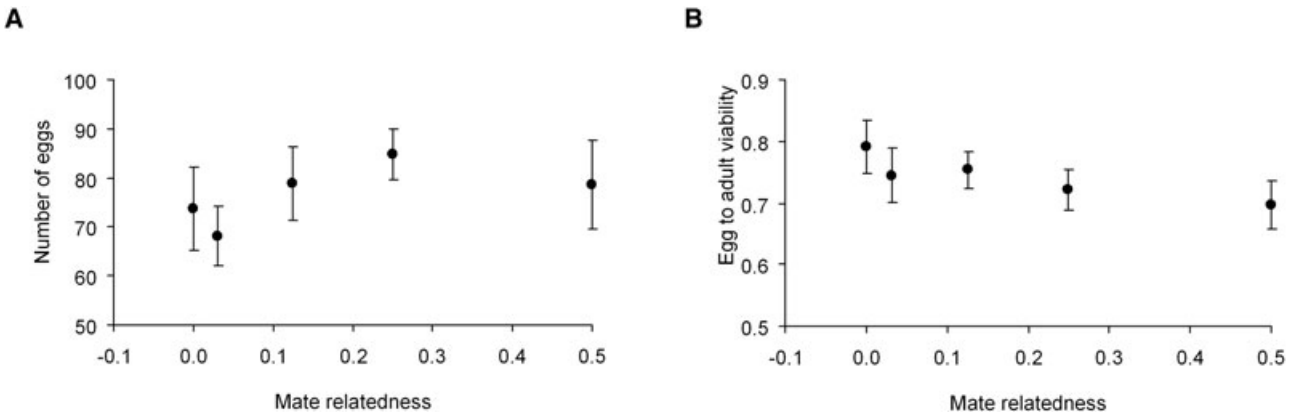

Figure 2. Mean ± SE of reproductive trait estimates by degree of relatedness between focal females and male mates in the parental generation. The number of eggs laid (A) and the egg-to-adult viability (B) are shown.

(Deviance = 28.4, $P = 0.130$ and Deviance = 2.0, $P = 0.640$ for the number of eggs and egg-to-adult viability, respectively), and there was no evidence of higher egg number or viability at intermediate levels of relatedness (Fig. 2). Effect sizes for the nonsignificant linear and quadratic inbreeding effects were low (range = 0.004–0.037).

Offspring fitness

Female fitness

The level of inbreeding had no significant effect on the number of eggs laid by females of the F1 generation (Table 2). However, egg-to-adult viability of their offspring (F2) showed a significant linear decrease with increasing inbreeding in the F1 female parent (Fig. 3; Table 2). As with the parental generation, the addition of a quadratic term did not significantly improve the fit of either GLM model (Deviance = 1.0, $P = 0.840$ and Deviance = 14.6, $P = 0.330$ for the number of eggs and egg-to-adult viability, respectively) and there was no evidence of higher egg number or viability at intermediate levels of inbreeding (Fig. 3A, B). Effect sizes for the nonsignificant quadratic terms were consistently low (range = 0.001–0.028).

Male fitness

The level of inbreeding had no significant effect on the ability of males to defend against their sperm being displaced by a competitor (P1), the ability of males to displace a competitor's sperm (P2), or the number of eggs produced when crossed with an unrelated female (Table 2). There was also no improvement of model fit with the addition of a quadratic inbreeding term for any of these traits (Deviance = 36.1, $P = 0.230$; Deviance = 1.5, $P = 0.760$; and Deviance = 12.0, $P = 0.510$ for P1, P2 and the number of eggs produced when

Table 2. The effect of inbreeding on reproductive fitness traits in the offspring generation

Trait	Source	d.f.	Deviance	*F*	*P*			η_p^2
					Model 1	Model 2	Randomization	
Females								
Eggs laid by F1 females	Block	11	432.4	1.63	0.132	0.101	0.089	0.340
	Inbreeding coefficient	1	16.8	0.70	0.408	0.381	0.382	0.018
	Block × Inbreeding coefficient	11	311.0	1.18	0.338	0.304	0.306	0.254
	Total	58	1671.5					
Egg-to-adult viability of eggs	Block	11	445.8	2.68	**0.013**	**0.011**	**0.012**	0.266
produced by F1 females*	Inbreeding coefficient	1	72.7	4.81	**0.035**	**0.035**	**0.043**	0.056
	Block × Inbreeding coefficient	11	169.9	1.02	0.448	0.496	0.567	0.121
	Total	58	1230.4					
Males								
P1	Block	11	516.6	1.84	0.086	0.096	0.073	0.232
	Inbreeding coefficient	1	25.5	1.00	0.324	0.381	0.378	0.015
	Block × Inbreeding coefficient	11	331.9	1.18	0.335	0.244	0.279	0.162
	Total	56	1713.2					
P2	Block	11	630.6	3.64	**0.002**	**0.008**	**0.013**	0.305
	Inbreeding coefficient	1	15.3	0.97	0.331	0.387	0.401	0.011
	Block × Inbreeding coefficient	11	210.6	1.22	0.316	0.438	0.500	0.128
	Total	56	1438.6					
Eggs laid by females	Block	11	785.8	2.62	**0.015**	**0.006**	**0.004**	0.281
Mated to F1 males	Inbreeding coefficient	1	0.4	0.01	0.907	0.899	0.893	< 0.000
	Block × Inbreeding coefficient	11	123.0	0.41	0.942	0.896	0.911	0.058
	Total	58	2007.8					
Egg-to-adult viability of	Block	11	323.5	2.50	**0.020**	**0.020**	**0.037**	0.271
Eggs fertilized by F1 males	Inbreeding coefficient	1	51.8	4.41	**0.043**	**0.046**	0.055	0.056
	Block × Inbreeding coefficient	11	91.1	0.70	0.726	0.671	0.634	0.095
	Total	57	870.3					

The results were analysed with two models. Model 1 is a generalized linear model with a quasibinomial (proportional data) or quasipoisson (count data) error using sequential deletion for statistical inference. Model 2 presents probability values from a mixed model analysis of variance. Model 2 was also tested with a permutation test (1000 replicates). Significant terms are highlighted in bold. *Residuals not normally distributed after arcsine transformation.

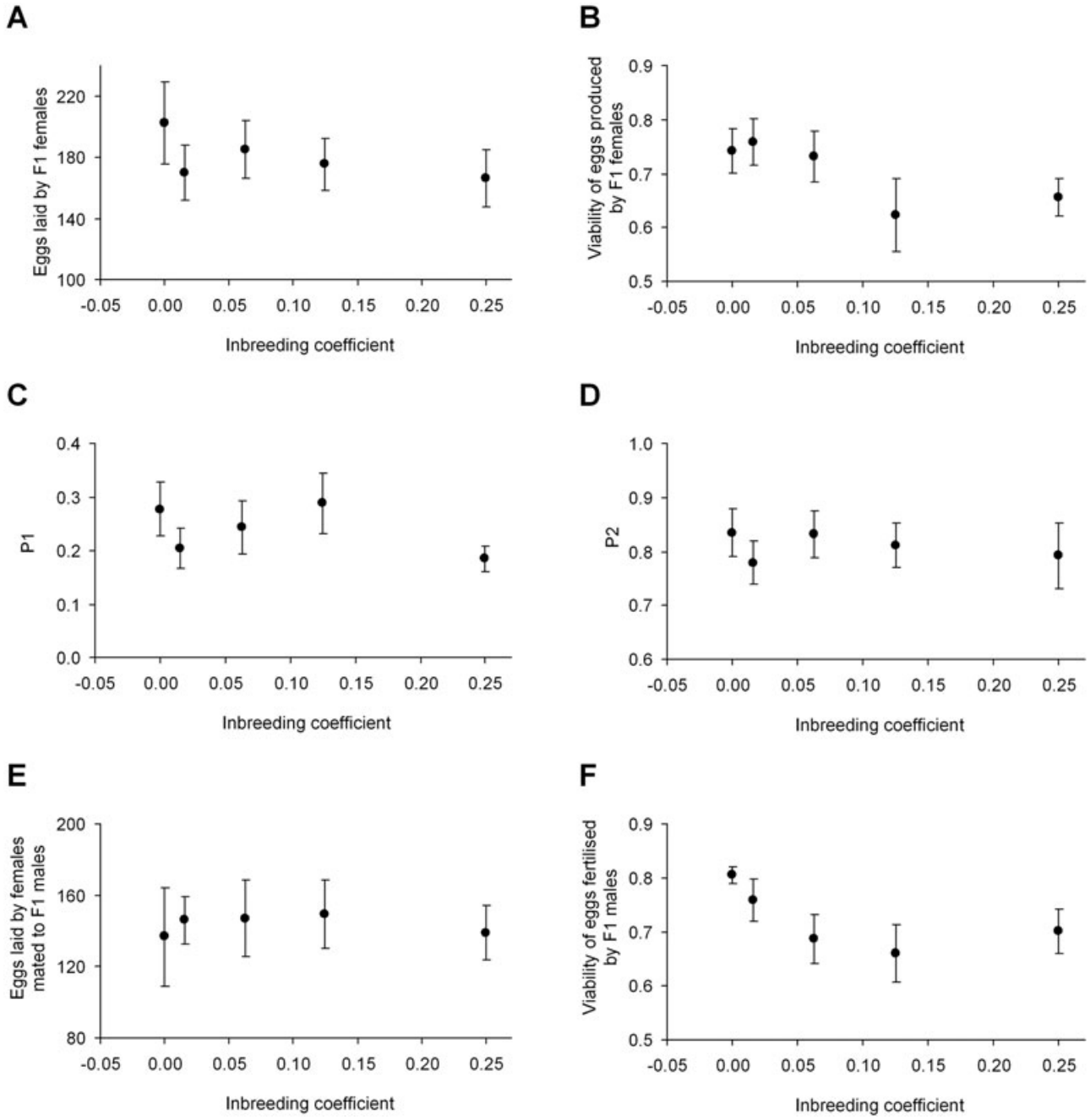

Figure 3. Mean ± SE of reproductive trait estimates by degree of inbreeding in the offspring generation. The number of eggs laid by F1 females (A), egg-to-adult viability of eggs produced by F1 females (B), P1 (C), P2 (D), the number of eggs laid by females mated to F1 males (E), and egg-to-adult viability of eggs fertilized by F1 males (F) are shown.

crossed to an unrelated female, respectively), and there was no evidence of higher fitness with intermediate levels of inbreeding (Fig. 3C, D, E). As with female fitness, effect sizes for the nonsignificant linear and quadratic inbreeding effects were low, in the range 0.026–0.029 for the linear inbreeding effect and 0.003–0.043 for the nonlinear inbreeding effects.

By contrast to the other male fitness traits, egg-to-adult viability of F2 offspring showed a significant linear decrease with increasing inbreeding in the F1 male parent (Fig. 3F; Table 2). Adding a quadratic term to this linear model significantly improved the fit (Deviance = 101.0, $P < 0.001$), although the relationship was opposite to that expected with optimal

outbreeding, with intermediate levels of inbreeding in F1 males showing the lowest F2 egg-to-adult viability (Fig. 3F).

To take into account the effect of inbreeding on egg-to-adult viability of progeny derived from inbred male flies, we adjusted our sperm competition progeny counts by dividing the number of wild-type offspring by the mean egg-to-adult viability for each level of inbreeding. We used these adjusted counts to calculate an adjusted P1 and P2. Note that, because the average viability of competitor (bw^D) males was not measured, we could not adjust the number of bw^D offspring, and so our adjustment overestimates the true values of P1 and P2 (but not the relative differences between them). Analysis of these data revealed very similar results to those found using the unadjusted estimates, and are available in the Supporting Information (Table S1).

DISCUSSION

The present study manipulated the level of inbreeding experimentally to test for continuous variation in its effects on the reproductive fitness of *D. melanogaster*. Because, in all cases except crosses between full siblings, our pedigree did not produce inbreeding of the X chromosome, these results represent a conservative estimate of inbreeding on female traits. This will not apply to male traits because males only have one copy of the X chromosome, meaning that inbreeding of the X chromosome does not occur in males.

The traits measured responded to inbreeding in different ways. For example, egg-to-adult viability demonstrated a linear decline with increased inbreeding, consistent with classical inbreeding depression, whereas the number of eggs laid and sperm competitive ability were unaffected. Only one trait, egg-to-adult viability of the progeny of F1 males, had a nonlinear relationship with the level of inbreeding. However, the shape of the relationship was opposite to that expected under optimal outbreeding: egg-to-adult viability was lowest at intermediate levels of inbreeding. The effect sizes for nonsignificant linear and quadratic terms were consistently low. The present study therefore provides little evidence of there being a fitness advantage to matings between individuals of intermediate relatedness.

Our experimental design allowed the effects of inbreeding on egg-to-adult viability to be examined in both the parent and offspring generations separately: F1 offspring derived from the parental crosses were inbred, but the F2 offspring derived from the F1 generation were not themselves inbred. Consistent with previous studies that suggest egg-to-adult viability in *Drosophila* is influenced by the genotypes of the embryo and mother (Rodriguez-Ramilo *et al.*, 2004), we found that inbred parents, and their offspring, had offspring with significantly reduced egg-to-adult viability. Furthermore, we found that F2 offspring egg-to-adult viability was affected by F1 parental inbreeding in both males and females. These results cannot be accounted for by inbreeding depression in the F2 embryos themselves because the F1 parents in these crosses were unrelated: inbred F1 males and F1 females were crossed to unrelated flies for both assays.

The effect of inbreeding on egg-to-adult viability in females could arise if inbred females laid a higher proportion of unfertilized eggs, so that they appeared to have reduced egg-to-adult viability. Similarly, inbred males may suffer reduced sperm quality and be less effective at fertilizing eggs. Under this hypothesis, females mated to inbred males should lay a greater proportion of unfertilized eggs, and will therefore appear to have reduced egg-to-adult viability.

A number of studies have linked inbreeding to reduced sperm quality giving support to this idea. For example, a study of bulb mites, *Rhizoglyphus robini*, found that the offspring of full sibling matings suffered reduced sperm competitive ability, presumably from reduced sperm quality (Konior *et al.*, 2005). Furthermore, Gage *et al.* (2006) found that reduced heterozygosity in wild rabbits, *Oryctolagus cuniculus* (presumably caused by inbreeding), was correlated with a reduction in testis size, and an increase in the proportion of abnormal sperm, an effect that may be widespread among mammals (Fitzpatrick & Evans 2009). Sperm quality has been shown to be an important factor in both defensive and offensive competitive fertilization success (Civetta, Rosing & Fisher, 2008).

However, by contrast to the findings with bulb mites (Konior *et al.*, 2005), the results of the P1 and P2 assays indicate that inbreeding, over the levels tested in the present study, does not affect the ability of a male to compete in offensive or defensive sperm competition. Our P1 and P2 results contrast those of Hughes (1997) who found evidence of an inbreeding decline in both P1 and P2 in *D. melanogaster*. However, the level of inbreeding imposed in Hughes' study was extreme (i.e. males were made homozygous for the entire third chromosome), so the difference between the two studies is not necessarily contradictory. Differences in the intensity of inbreeding may also explain why other traits that we examined showed little evidence of a linear decline with increased inbreeding, even though they have been shown to be affected by inbreeding depression previously (Lynch & Walsh, 1998). Because inbreeding did not appear to reduce sperm quality in the present study, the effects of inbreeding on the egg-to-adult

viability of offspring from inbred males are unlikely to be explained by fertilization failure.

An alternative explanation to fertilization failure for the effect of inbreeding on egg-to-adult viability that we observed in the present study is that inbreeding affects the direct environmental contribution that parents make to eggs. Although maternal effects might explain the result in females, it is less obvious how paternal effects from F1 males might influence egg-to-adult viability because, in *Drosophila*, females are not considered to receive material benefits from mating apart from receiving sperm (Chapman, Trevitt & Partridge, 1994; Pitnick, Spicer & Markow, 1997). A factor that could be producing the decline in egg-to-adult viability of offspring from inbred F1 males is the 133 seminal fluid proteins (SFPs), which male *Drosophila* transfer to females during mating (Swanson *et al.*, 2001; Wolfner, 2002; Findlay *et al.*, 2008). These SFPs have a positive influence on female short-term reproductive physiology via their effects on female receptivity, food intake, oogenesis, and ovulation (Ram & Wolfner, 2007). In crickets, *Teleogryllus oceanicus*, SFPs appear to influence embryo viability (Garcia-Gonzalez & Simmons, 2007). If the effects of SFP on female reproductive effort similarly determine embryo viability in *Drosophila*, the paternal effect that we observed could be explained by the effect of inbreeding on the quantity and/or quality of SFPs transferred by F1 males.

The presemt study has shown that linear declines in fitness are associated with increased inbreeding for some, but not all reproductive traits in *D. melanogaster*. Although some of the unaffected traits have shown inbreeding depression in previous studies, the levels of inbreeding were often extreme, and so the biological significance of these effects for natural populations is unclear. Our data provide estimates of the strength of fitness effects for levels of inbreeding possible in natural populations. We found no evidence for optimal fitness at intermediate levels of inbreeding for any trait that we examined. Although we found no evidence of a direct benefit to fitness, it should be noted that the present study did not examine any indirect fitness effects, such as inclusive fitness. Future studies need to consider the effects of biologically realistic levels of inbreeding, across multiple generations, when trying to understand the costs and benefits of mating with close relatives and may also need to take into account the effects on inclusive fitness.

ACKNOWLEDGEMENTS

We thank Dr Paco Garcia-Gonzalez for additional help with respect to the manuscript, Professor Mike Johnson for the use of laboratory facilities and controlled temperature rooms, Professor Bill Rice for kindly making available the LH_M-bw competitor stock, and Howard Park winery for allowing the collection of flies from their estate for establishing the stock population. The study was funded by the Australian Research Council and the University of Western Australia.

REFERENCES

Alipaz JA, Karr TL, Wu CI. 2005. Evolution of sexual isolation in laboratory populations: fitness differences between mating types and the associated hybrid incompatibilities. *American Naturalist* **165:** 429–438.

Barnard CJ, Fitzsimons J. 1988. Kin recognition and mate choice in mice – the effects of kinship, familiarity and social interference on intersexual interaction. *Animal Behaviour* **36:** 1078–1090.

Barnard CJ, Fitzsimons J. 1989. Kin recognition and mate choice in mice: fitness consequences of mating with kin. *Animal Behaviour* **38:** 35–40.

Bateson P. 1980. Optimal outbreeding and the development of sexual preference in the Japanese quail. *Zeitschrift fur Tierpsychologie* **53:** 231–244.

Bateson P. 1983. Optimal outbreeding. In: Bateson P, ed. *Mate choice*. Cambridge: Cambridge University Press.

Boorman E, Parker GA. 1976. Sperm (ejaculate) competition in *Drosophila melanogaster*, and reproductive value of females to males in relation to female age and mating status. *Ecological Entomology* **1:** 145–155.

Borrell YJ, Pineda H, McCarthy I, Vazquez E, Sanchez JA, Lizana GB. 2004. Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, *Salmo salar*. *Heredity* **92:** 585–593.

Byrne PG, Rice WR. 2005. Remating in *Drosophila melanogaster*: an examination of the trading-up and intrinsic male-quality hypotheses. *Journal of Evolutionary Biology* **18:** 1324–1331.

Carr DE, Dudash MR. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **358:** 1071–1084.

Chapco W. 1979. Correlations between chromosome segments and fitness in *Drosophila melanogaster*. II. The chromosome and egg viability. *Genetics* **92:** 595–601.

Chapman T, Davies SJ. 2004. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides* **25:** 1477–1490.

Chapman T, Trevitt S, Partridge L. 1994. Remating and male-derived nutrients in *Drosophila melanogaster*. *Journal of Evolutionary Biology* **7:** 51–69.

Charlesworth B, Charlesworth D. 1999. The genetic basis of inbreeding depression. *Genetical Research* **74:** 329–340.

Civetta A, Rosing KR, Fisher JH. 2008. Differences in sperm competition and sperm competition avoidance in *Drosophila melanogaster*. *Animal Behaviour* **75:** 1739–1746.

Clark AG, Aguade M, Prout T, Harshman LG, Langley CH. 1995. Variation in sperm displacement and its associa-

tion with accessory-gland protein loci in *Drosophila melanogaster*. *Genetics* **139:** 189–201.

Cohen LB, Derhorn DC. 2004. Great frigatebirds, *Fregata minor*, chose mates that are genetically similar. *Animal Behaviour* **68:** 1229–1236.

Crawley MJ. 2002. *Statistical computing: an introduction to data analysis using S-Plus*. Chichester: Wiley.

Findlay GD, Yi X, MacCoss MJ, Swanson WJ. 2008. Proteomics reveals novel *Drosophila* seminal fluid proteins transferred at mating. *PLoS Biology* **6:** e178.

Fitzpatrick JL, Evans JP. 2009. Reduced heterozygosity impairs sperm quality in endangered mammals. *Biology Letters* **5:** 320–323.

Gage MJG, Surridge AK, Tomkins JL, Green E, Wiskin L, Bell DJ, Hewitt GM. 2006. Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. *Current Biology* **16:** 612–617.

Garcia N, Lopez-Fanjul C, Garcia-Dorado A. 1994. The genetics of viability in *Drosophila melanogaster*: effects of inbreeding and artificial selection. *Evolution* **48:** 1277–1285.

Garcia-Gonzalez F, Simmons LW. 2007. Paternal indirect genetic effects on offspring viability and the benefits of polyandry. *Current Biology* **17:** 32–36.

Gilchrist AS, Partridge L. 1999. A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. *Genetics* **153:** 1775–1787.

Goldstein LSB, Fyrberg EA. 1994. *Drosophila melanogaster: practical uses in cell and molecular biology*. San Diego, CA: Academic Press.

Greaves JH, Redfern R, Ayres PB, Gill JE. 1977. Warfarin resistance: a balanced polymorphism in the Norway rat. *Genetical Research* **30:** 257–263.

Helgason A, Pálsson S, Guðbjartsson DF, Kristjánsson Þ, Stefánsson K. 2008. An association between the kinship and fertility of human couples. *Science* **319:** 813–816.

Hughes KA. 1997. Quantitative genetics of sperm precedence in *Drosophila melanogaster*. *Genetics* **145:** 139–151.

Kalb JM, DiBenedetto AJ, Wolfner MF. 1993. Probing the function of *Drosophila melanogaster* accessory glands by directed cell ablation. *Proceedings of the Royal Society of London Series B, Biological Sciences* **90:** 8093–8097.

Keane B. 1990. The effect of relatedness on reproductive success and mate choice in the white-footed mouse, *Peromyscus leucopus*. *Animal Behaviour* **39:** 264–273.

Keller LF, Waller DM. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution* **17:** 230–241.

Kokko H, Ots I. 2006. When not to avoid inbreeding. *Evolution* **60:** 467–475.

Konior M, Keller L, Radwan J. 2005. Effect of inbreeding and heritability of sperm competition success in the bulb mite, *Rhizoglyphus robini*. *Heredity* **94:** 577–581.

Levine TR, Hullett CR. 2002. Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research* **28:** 612–625.

Lopez-Fanjul C, Villaverdea A. 1989. Inbreeding increases genetic variance for viability in *Drosophila melanogaster*. *Evolution* **43:** 1800–1804.

Lynch M, Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer Associates.

Mack PD, Hammock BA, Promislow DEL. 2002. Sperm competitive ability and genetic relatedness in *Drosophila melanogaster*: similarity breeds contempt. *Evolution* **56:** 1789–1795.

Partridge L. 1983. Non-random mating and offspring fitness. In: Bateson P, ed. *Mate choice*. Cambridge: Cambridge University Press.

Pitnick S, Spicer GS, Markow T. 1997. Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. *Evolution* **51:** 833–845.

Ram KR, Wolfner MF. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. *Integrative and Comparative Biology* **47:** 427–445.

Rodriguez-Ramilo ST, Perez-Figueroa A, Fernandez B, Fernandez J, Caballero A. 2004. Mutation-selection balance accounting for genetic variation for viability in *Drosophila melanogaster* as deduced from an inbreeding and artificial selection experiment. *Journal of Evolutionary Biology* **17:** 528–541.

Swanson WJ, Clark AG, Waldrip-Dail HM, Wolfner MF, Aquadro CF. 2001. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 7375–7379.

Templeton AR. 1986. Coadaption and outbreeding depression. In: Soule ME, ed. *Conservation biology: the science of scarcity and diversity*. Sunderland, MA: Sinauer Associates.

Wolfner MF. 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88:** 85–93.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. The effect of inbreeding on sperm competitive ability in the offspring generation; corrected for mean egg-to-adult viability within each level of inbreeding.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.