THE ROYAL SOCIETY

Does interspecific hybridization influence evolutionary rates? An experimental study of laboratory adaptation in hybrids between *Drosophila serrata* and *Drosophila birchii*

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The low initial fitness of progeny from interspecific crosses in animals and the rarity of interspecific hybridization in natural environments have led to a debate about the evolutionary importance of this phenomenon. Here we directly assess the effects of hybridization between *Drosophila serrata* and *Drosophila birchii* on evolutionary rates. We looked at the effects on laboratory adaptation over 30 generations in two laboratory environments, one of which involved nutrition and temperature stress. Laboratory adaptation occurred over time in both environments as reflected by a marked change in viability. However, whilst hybrid lines at no stage performed poorly relative to parental lines, their rate of adaptation never exceeded that of the parentals. Thus, there was no evidence that hybridization increased evolutionary rates. Instead, hybrid lines converged phenotypically with one of the parental species.

Keywords: hybrids; *Drosophila*; evolution; viability selection; laboratory adaptation

1. INTRODUCTION

In recent years there has been renewed interest in the process of natural interspecific hybridization and its evolutionary importance. It is generally accepted that hybridization occurs in natural populations (Arnold 1997; Coyne & Orr 1998; Rieseberg & Carney 1998). Reviews of the fossil record and extant populations using trait morphology and genetics (e.g. Levin 1979; Rieseberg 1995; Arnold 1997) suggest that hybridization is extensive particularly in plants. The frequency of hybridization in animals is thought to be lower (Arnold 1997), but molecular approaches suggest that it occurs more often than previously thought (Bullini 1994; Arnold *et al*. 1999).

Hybridization and introgression are thought to play a role in evolution because they lead to novel genotypes (Anderson & Stebbins 1954; Arnold 1997). In plants, this process is considered particularly important (Lotsy 1925; Anderson & Stebbins 1954) and may have contributed to past diversification during environmental changes (see Cruzan & Arnold 1993; Rieseberg *et al*. 1996; Fritz 1999). In animals, interspecific hybridization is usually considered an evolutionary dead end because crosses often result in no progeny or individuals with no fertility or reduced viability (e.g. Dobzhansky 1937; Mayr 1963) and data demonstrating an effect of interspecific hybridization on evolutionary rates are sparse. In a widely cited study, Lewontin & Birch (1966) used phenotypic classes constructed through hybridizing species in the laboratory to assess the distribution of colour variants in *Dacus* fruitflies in nature. These experiments suggested that genetic variation allowing *Dacus tryoni* to increase its environmental tolerance and range came from *Dacus neohumeralis* genes introgressed following hybridization. However, this was never directly demonstrated. In Hawaiian *Drosophila*, Carson *et al*. (1975) found evidence for gene exchange between two species in a disturbed habitat suggesting that hybrid genotypes may have facilitated adaptation. In Galápagos Island finches (*Geospiza* spp.), hybrid individuals appear to have higher fitness than parental species and occur relatively more often during unusual stressful conditions (Grant & Grant 1996).

While these data argue indirectly for a role of hybridization in animal evolution, direct evidence is generally lacking. In particular, it is not clear whether hybridization increases evolutionary rates beyond those possible from variation within species. More direct evidence can be obtained from experimental evolution where the impact of hybridization can be directly compared with rates for parental species. Unfortunately, experimental animal studies on hybridization do not usually extend beyond a few generations (e.g. Shaw & Wilkinson 1980; Scribner 1993; Price & Boake 1995) whereas the long-term effects need to be considered for predicting evolutionary consequences (Rieseberg & Carney 1998).

Drosophila serrata and *Drosophila birchii* are Australian endemics presenting an ideal situation for looking at the effects of hybridization on experimental evolution. These species differ in distribution with regions of sympatry (Ayala 1965*a*; Bock 1976) and differ in both life-history and stress resistance traits (Hoffmann 1991; Berrigan & Hoffmann 1998; Hercus & Hoffmann 1999). While hybridization is often asymmetrical in *Drosophila* (Wu & Beckenbach 1983; Orr & Coyne 1989), both reciprocal

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Received 13 July 1999 *Accepted* 28 July 1999

crosses between *D. serrata* and *D. birchii* produce fully fertile hybrids. In the laboratory, approximately one in 1000 crosses are successful (Ayala 1965*a*; Blows 1998). The resulting hybrid isofemale lines vary in their levels of resistance to desiccation, heat and cold stress (Berrigan & Hoffmann 1998; Hercus & Hoffmann 1999). Differences between reciprocal hybrids are maintained in hybrid lines founded from them suggesting that components of both parental genomes are maintained over time (Berrigan & Hoffmann 1998; Blows & Allan 1998; Hercus & Hoffmann 1999). *Drosophila serrata* and *D. birchii* have both previously been used in comparisons of the rates of experimental evolution in crosses between and within populations. In particular, Ayala (1965*b*) showed that lines derived from interpopulation crosses of *D. serrata* had greater variance, a higher productivity and an increased population size relative to intrapopulation crosses.

Here we extend these observations to the interspecific level by comparing evolution in hybrid and parental lines. To measure evolutionary rates, we focused on viability changes arising from laboratory adaptation. In previous work we found that fecundity and, in particular, egg–adult viability of lines of *D. birchii* and *D. serrata* increase with time in the laboratory (Hercus & Hoffmann 1999; M. J. Hercus, unpublished). Changes in the viability of mass-bred populations are highly repeatable and involve an increase of $>20\%$ after 30 generations. To investigate the effect of interspecific hybridization on this process, we generated hybrids between *D. serrata* and *D. birchii* and performed a direct comparison of the fitness of replicate hybrid and parental populations after 17 and 30 generations of laboratory rearing. Since hybridization effects may be more pronounced under stressful conditions, we compared hybrid and parental populations under normal laboratory rearing conditions as well as a more stressful laboratory environment involving poor nutrition and cold temperature exposures.

2. MATERIAL AND METHODS

(a) *Stocks*

Isofemale lines of *D. serrata* and *D. birchii* were generated from females collected from north-eastern Queensland in January 1997. Since females of *D. serrata* and *D. birchii* are indistinguishable, species identification was from male progeny in the following generation. Identification is from the number of bristles on the external genitalia. *Drosophila serrata* males always have two bristles on either side of the genital arch whilst *D. birchii* have three. Bristle morphology is presented as the number on the left side followed by the number on the right side (i.e. *D. serrata* 2–2 and *D. birchii* 3–3).

To generate the hybrid population, crosses were undertaken between isofemale lines in both directions. Five crosses were successful. These lines had recently ($F_{4–5}$) originated from females collected from Paluma, Kirrama, Eungella and Coffs Harbour (*D. serrata*) and Kirrama, Eungella and Mossman (*D. birchii*). F_1 bristle morphology was used for confirmation of hybrid status, with hybrids showing aberrant forms (2–3, 3–1, 1–4, etc.). All F_1 progeny ($n=219$) from the successful interspecific crosses were combined to produce a single set of hybrid F_2s which were then used to establish eight hybrid populations. At this stage an equivalent number of parental lines of the two species was also set up from the lines used to generate the hybrids. Thus, parental lines contained genotypes from several conspecific populations, while hybrid lines contained genetic variation from interspecific crosses as well as the intraspecific variation used to set up the parental lines.

Table 1. *Nested ANOVAs for viability using data from* D. serrata, D. birchii *and hybrid groups*

	mean squares	
factor (d.f.)	generation 17	generation 30
group (2)	2863.01***	1421.67**
rearing (1)	91.22	1045.91*
testing (1)	72 499.92***	39 809.47***
testing × rearing (1)	671.40*	1507.49**
testing × group (2)	112.22	603.04*
rearing × group (2)	268.15	117.17
line within (group × rearing) (9)	68.89	102.95
testing rearing × group (2)	61.91	213.31
testing × line within (group × rearing) (18)	109.62*	126.76
error (240)	67.35	127.27

* $p \leqslant 0.05$, ** $p \leqslant 0.01$, *** $p \leqslant 0.001$.

(b) *Experimental conditions*

Lines selected for the normal environment (the 'normal' lines) were reared on an agar–dead yeast–potato–sugar medium. For lines reared in the stressful environment (the 'stress' lines), a combination of stressful conditions involving low food quality and cold exposures was used. The low-quality medium had no potato and a reduced (25%) amount of yeast. In addition, the first and second instar larvae were exposed to three 90 min intervals at 1 °C. Pilot experiments indicated that the viability in these conditions for fresh stocks was 18% (± 2) for *D. serrata* and 11% (± 1) for *D. birchii* compared to 59% (± 1) and 50% (± 2), respectively, in the normal environment.

All stocks were maintained at 25 °C under continuous light in three standard culture bottles per line with a generation time of two to three weeks. All adults were allowed to eclose and held together before the next generation was initiated using 80 individuals per bottle.

(c) *Measurements*

After 17 and 30 generations of selection, the egg-to-adult viability was examined under both stressful and normal conditions. Eggs were collected by placing 70 pairs of F_{16} (or F_{29}) adults (per line) into an empty glass bottle and covering this with a watchglass containing 2 ml of treacle medium. This was covered with a yeast solution to stimulate oviposition. Flies were left for 10 h in darkness at 25 °C. Eggs (30 per vial) were transferred to six replicate vials per line each containing 13 ml of medium.

(d) *Data analysis and explanation of terms*

All data were arcsine transformed prior to analysis with nested ANOVAs involving four main factors in the design. The first of these is 'rearing', the environment in which the lines were reared (and to which they adapted) for the duration of the experiment. This consisted of the normal environment or the

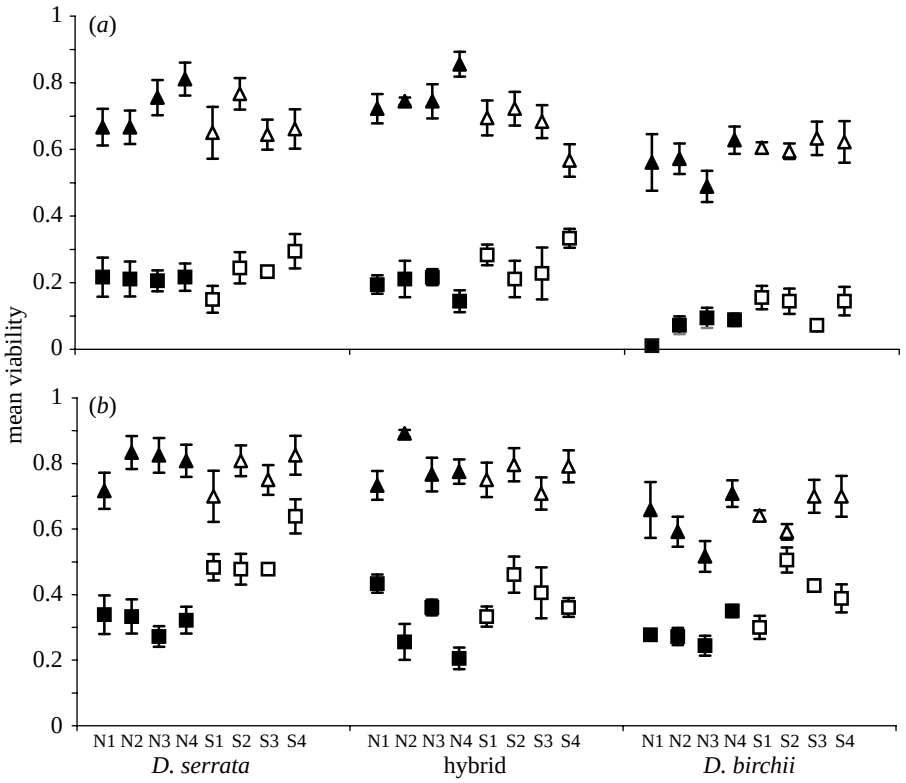

Figure 1. Mean viability for each replicate line of the *Drosophila serrata*, *Drosophila birchii* and hybrid groups measured in both testing environments. Viability was measured at (*a*) generation 17, and (*b*) generation 30. Lines reared under normal conditions (N1–N4) are represented by solid symbols and stress lines (S1–S4) by open symbols. Error bars represent the standard error of the means. Triangles, normal testing environment; squares, stressful testing environment.

stressful environment. The 'testing' term refers to the normal or stressful environment used in the direct comparison of the lines. 'Group' refers to the three groups of flies studied (*D. birchii*, *D. serrata* and hybrid) and 'line' refers to the nested line factor. Line is nested within both the group and rearing environments because four replicate lines were used for all groups in both rearing environments. Interactions were also investigated. In particular the rearing × group interaction tests whether group differences have been affected by the selection environment and the group × rearing × testing interaction indicates if any group differences depended on the testing environment.

3. RESULTS

(a) *Generation 17*

There were four significant effects in the ANOVA when all three groups and both testing and rearing environments were considered (table 1). The mean viability of all groups was lower in the stressful testing environment relative to the normal environment (figure 1), accounting for the testing effect. There was a group effect, partly due to the tendency of the *D. serrata* and hybrid populations to have higher viability than *D. birchii* in both environments. There was also a significant testing × rearing interaction. For two groups, the normal lines had a higher mean viability than the stress lines when tested in the normal environment, whilst in the stressful environment the stress lines from all groups had higher viability compared to their respective normal lines (figure 1). There was a suggestion that replicate lines within the groups behaved differently depending on where they were tested ($F_{18,240} = 1.63$ and $p = 0.05$).

The means for the hybrid and *D. serrata*, populations tended to be closer to one another than either was to *D. birchii* (see figure 1). An ANOVA was therefore undertaken on the hybrid and *D. serrata* data to examine the interactions further. The testing × rearing interaction remained significant in the reduced data set ($F_{1,12} = 5.39$ and $p = 0.04$) confirming the fact that lines adapted to one environment performed relatively better in that environment. However, none of the terms that included the group factor were significant, reflecting the fact that viability changes in the hybrids and *D. serrata* were similar.

(b) *Generation 30*

There were significant effects of testing, rearing and group in the ANOVA (table 1). The mean viability in the normal environment was relatively higher (figure 1) and, under normal testing conditions, the *D. serrata* and hybrid populations had a higher viability than the *D. birchii* populations. In stressful testing conditions, stress lines had higher viability than normal lines (testing × rearing interaction). There was also a significant testing × group effect. In the normal environment, the *D. birchii* populations had lower viability than the other groups but this was not as evident in the stressful environment.

When only the *D. serrata* and hybrid populations were compared, there was a significant testing × rearing interaction ($F_{1,12} = 11.65$ and $p = 0.005$) confirming the environment-specific nature of the adaptation response. However, the testing × group interaction was no longer significant ($F_{1,12} = 1.16$ and $p = 0.303$) because the different responses of the groups to the environments were due to *D. birchii*.

(c) *Comparisons within groups*

We also performed nested ANOVAs for the three groups separately to examine the effects of replicate lines and rearing environment in more detail (table 2). The proportional contribution of each factor to the variance in the viability scores is presented. When the stress and

Table 2. *Mean squares from nested ANOVAs considering the replicate lines for each group separately*
(The contribution of each factor towards the total variance is also given.)

	mean squares		proportion of variance	
factor (d.f.)	generation 17	generation 30	generation 17	generation 30
hybrid lines				
rearing	13.14	40.11	0.001	0.002
testing (1)	21 952.30***	188 892.22***	0.964	0.956
line within rearing (6)	16.98	88.49	0.001	0.005
testing × rearing (1)	573.57	370.78	0.025	0.019
testing × line within rearing (1)	147.78*	234.48	0.006	0.012
error (80)	59.82	128.41	0.003	0.007
D. serrata lines				
rearing (1)	17.79	534.09	0.001	0.032
testing (1)	22 563.78***	14 229.16***	0.982	0.859
line within rearing (6)	120.74	146.86	0.005	0.009
testing × rearing (1)	114.53	1470.21**	0.005	0.088
testing × line within rearing (6)	75.50	50.27	0.003	0.003
error (80)	81.46	132.97	0.004	0.008
D. birchii lines				
rearing (1)	597.75*	706.06	0.021	0.078
testing (1)	28 223.23***	7894.17***	0.967	0.872
line within rearing (6)	84.90	144.43	0.003	0.016
testing × rearing (1)	103.26	93.13	0.004	0.010
testing × line within rearing (6)	105.39	95.51	0.004	0.011
error (80)	60.67	120.45	0.002	0.013

* $p \leqslant 0.05$, ** $p \leqslant 0.01$, *** $p \leqslant 0.001$.

normal hybrid lines were compared at generation 17 there was a significant effect of testing × line. Two replicate stress lines had higher viability in the stressful testing environment than the normal lines (figure 1). This interaction was not evident at generation 30. None of the other factors were significant, including the interaction between testing and rearing which would have indicated environment-specific adaptation. For *D. serrata*, a significant testing × rearing effect was evident at generation 30, because lines reared under stressful conditions had a relatively higher fitness when tested under these conditions (figure 1). For the *D. birchii* lines, there was a significant rearing effect at F_{17} because the viability of the stress lines was relatively higher regardless of testing conditions. Replicate lines of *D. serrata* and *D. birchii* acted in a consistent manner across the testing environments, because there was no significant effect of line in the ANOVAs. These data suggest that hybrid lines behaved differently at generation 17 in their response to the two environments whereas parental species lines behaved similarly. However, the proportion of the variance accounted for by line effects (or line × testing interaction effects) was not particularly high even in the hybrids.

(d) *Changes in viability between generations 17 and 30*

To examine the changes that occurred over time in the groups, we compared the measures of viability obtained for each replicate line at generations 17 and 30 (after arcsine transformation). These measures are presented in figure 2 and indicate that lines had a higher viability in generation 30 than generation 17 regardless of their rearing and testing environment. An ANOVA indicates a significant interaction between group and testing environment ($F_{2,36} = 6.07$ and $p < 0.01$) as well as a significant testing environment effect ($F_{1,36} = 33.22$ and $p < 0.001$). Under normal testing conditions, the adaptive response of the three groups tended to be similar ($F_{2,18} = 0.76$ and $p = 0.48$) and there is no effect of rearing environment ($F_{1,18} = 0.18$ and $p = 0.68$) or an interaction between these factors ($F_{2,18} = 0.23$ and $p = 0.80$). Under stressful testing conditions, the greatest changes in the mean viability occurred in *D. birchii*. The differences between the groups were significant ($F_{2,18} = 7.79$ and $p < 0.01$) although there was no overall effect of rearing ($F_{1,18} = 2.42$ and $p = 0.14$) and the interaction between these factors was marginally non-significant ($F_{2,18} = 2.68$ and $p < 0.10$). Thus, there was a difference between the groups in the rate of adaptation under stressful conditions between generations 17 and 30, but this involved a more rapid response to selection in the *D. birchii* lines rather than the hybrids.

4. DISCUSSION

The results indicate that all groups adapted to the two types of environment. The viability at generation 30 was higher than at generation 17 and the viability at generation 17 was higher than that observed when the stocks of *D. serrata* and *D. birchii* were first established. This increase was particularly marked under stressful conditions. Pilot experiments indicated that the viability of the parental species was originally less than 20% whereas this had increased to *ca.* 50% in some lines by the end of the experiment. The environment-specific response tended to be overshadowed by increases in the viability in all lines in both environments. Lines adapted to normal conditions

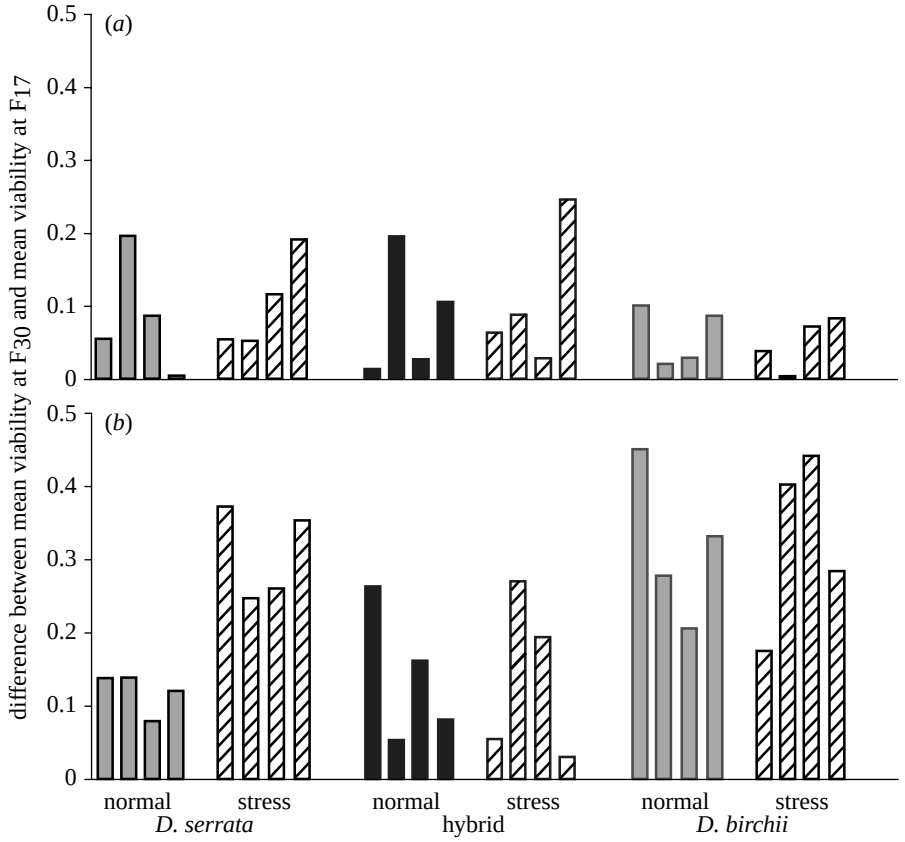

Figure 2. Increase in viability observed between generations 17 and 30 in the lines when measured in both testing environments: (*a*) normal conditions, and (*b*) stressful testing conditions. For each group (*Drosophila serrata*, *Drosophila birchii* or hybrids), normal lines are indicated by solid bars and stress lines by hatched bars.

also showed increased viability under stressful conditions and vice versa.

The hybrid lines performed well compared to those of their parental species, but there was no evidence that hybridization increased evolutionary rates. After 17 generations, the hybrid lines were performing as well as *D. serrata* and better than the *D. birchii* lines. However, there was no evidence that the magnitude of the adaptive response differed between the groups at either generation. This includes both the general response and the environment-specific response. The only difference between groups that we detected involved a relatively more rapid increase in the viability of the *D. birchii* lines between generations 17 and 30.

It is possible that inbreeding influenced our results. A higher level of inbreeding in the hybrids may have prevented a more rapid adaptive shift. Inbreeding may be higher in hybrid lines because of a breakdown in the mate recognition system, reducing the number of successful matings. However, Blows & Allan (1998) found that crosses among hybrid individuals from the same line were generally successful. We evaluated the effect of inbreeding directly by observing the performance of crosses between and within lines of the *D. serrata* and hybrid groups. There were no viability differences between crosses within lines and between lines of *D. serrata* ($F_{1,4}=0.02$ and $p=0.88$) and the hybrid lines ($F_{1,4}=1.78$ and $p=0.25$). Thus, inbreeding effects appear to be absent.

While our experimental approach provides a direct way of investigating hybridization effects, there are several limitations. Arnold & Hodges (1995) considered it important to subdivide recombinant groups arising from hybridization into many classes because there is often variation between them. Here we started with five hybridization events and pooled the progeny from reciprocal cross types rather than setting up separate lines. This would have reduced the variance between hybrid lines, although any novel hybrid genotypes would have been directly exposed to viability selection. Another limitation is that the environments tested may not have been sufficiently novel. The changes in viability over time indicate that the parental species can adapt to the conditions we used. Hybridization effects might be more apparent when parental species fail to adapt to the experimental conditions. Finally, hybridization effects may have been detected in species that are homosequential across the genome, whereas *D. birchii* and *D. serrata* carry some inversions (e.g. Baimai 1970) and these may differ between the species.

The data suggest that the hybrids converged rapidly on the phenotype of *D. serrata*. Such convergence has also been noted in other studies. Nagy (1997) found that, in plants, native characters were favoured over character states generated through hybridization. Observations on Louisiana iris hybrids in nature (Cruzan & Arnold 1993) suggested that hybrid genotypes tend to be more similar to parentals as a result of selection and assortative mating which reduce the frequency of intermediate types. A recent mussel study (Wilhelm & Hilbish 1998) found strong viability selection among hybrid genotypes which resulted in the near elimination of genotypes originating from one parent. Finally, in molecular studies on *Helianthus annus* and *Helianthus petiolaris* hybrids (Rieseberg *et al*. 1996; Rieseberg & Linder 1999) all hybrids had fewer *H. petiolaris* markers than expected and variation was not high, individuals from three hybrid lineages converging to similar combinations of genes. Despite this, hybrids from crosses between *D. serrata* and *D. birchii* do not converge to the *D. serrata* phenotype for

all traits; other data show that hybrids can be similar to either parent or intermediate (Berrigan & Hoffmann 1998; Hercus & Hoffmann 1999).

In conclusion, we found that hybrids are not generally at a disadvantage relative to parental species, but there is also no evidence that interspecific hybridization increased the rates of evolutionary change. The variability generated from combining the genotypes of two different species therefore did not increase the rates of adaptive change in response to two laboratory environments when compared to the variability available within the parentals and despite the fact variation is generated from the hybridization event we considered (Hercus & Hoffmann 1999).

This research was supported by a grant from the Australian Research Council to A.A.H. We are grateful to two anonymous reviewers for comments on the manuscript.

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