

# Genotype-by-environment interactions leads to variable selection on life-history strategy in Common Evening Primrose (*Oenothera biennis*)

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## Abstract

Monocarpic plant species, where reproduction is fatal, frequently exhibit variation in the length of their prereproductive period prior to flowering. If this life-history variation in flowering strategy has a genetic basis, genotype-by-environment interactions ( $G \times E$ ) may maintain phenotypic diversity in flowering strategy. The native monocarpic plant Common Evening Primrose (*Oenothera biennis* L., Onagraceae) exhibits phenotypic variation for annual vs. biennial flowering strategies. I tested whether there was a genetic basis to variation in flowering strategy in *O. biennis*, and whether environmental variation causes  $G \times E$  that imposes variable selection on flowering strategy. In a field experiment, I randomized more than 900 plants from 14 clonal families (genotypes) into five distinct habitats that represented a natural productivity gradient.  $G \times E$  strongly affected the lifetime fruit production of *O. biennis*, with the rank-order in relative fitness of genotypes changing substantially between habitats. I detected genetic variation in annual vs. biennial strategies in most habitats, as well as a  $G \times E$  effect on flowering strategy. This variation in flowering strategy was correlated with genetic variation in relative fitness, and phenotypic and genotypic selection analyses revealed that environmental variation resulted in variable directional selection on annual vs. biennial strategies. Specifically, a biennial strategy was favoured in moderately productive environments, whereas an annual strategy was favoured in low-productivity environments. These results highlight the importance of variable selection for the maintenance of genetic variation in the life-history strategy of a monocarpic plant.

## Introduction

Variable selection in space and time can maintain genetic variation and give rise to a diversity of life-history strategies within populations (Levins, 1968; Roff, 1992; Stearns, 1992; Byers, 2005). Several conditions must be satisfied for this to occur. First, there must be heterogeneity in the abiotic or biotic environment. Second, genotype and environment must interact to affect com-

ponents of fitness, whereby the rank-order in the mean fitness of genotypes changes between environments (Mitchell-Olds, 1992). And last, genetic variation in fitness must correlate with genetic variation in life-history traits (Cole, 1954; Hart, 1977; Van Tienderen, 1997). Here, I ask whether spatial variation along a natural environmental gradient causes genotype-by-environment interactions ( $G \times E$ ) that lead to divergent selection on genetic variation in the flowering life-history strategy of a native plant.

Monocarpic plant species, where reproduction is fatal, are ideally suited for the study of life-history evolution in flowering strategy. The age at first reproduction, the number of reproductive bouts and lifespan are important

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life-history traits that together depict a plant's flowering strategy (Stearns, 1976; Metcalf *et al.*, 2003). Monocarpic plant species frequently exhibit variation in the length of their prereproductive period, from one (annuals), to two (biennials), or more growing seasons (Metcalf *et al.*, 2003). Such variation is typically caused by individual differences in vernalization requirements and the threshold size required to flower (Boudry *et al.*, 2002; Metcalf *et al.*, 2003; Stinchcombe *et al.*, 2005). Evidence suggests that there is a genetic basis to variation in whether a plant is annual, biennial or longer-lived (Smith, 1927; Lacey, 1986; Simons & Johnston, 2000). However, most studies of natural populations have focused on variation over large latitudinal gradients (Reinartz, 1984; Lacey, 1988; Boudry *et al.*, 2002), whereas relatively little is known about genetic variation within populations and the microevolutionary processes that maintain variation in flowering strategy (Law *et al.*, 1977; Donohue, 2002).

Life-history theory offers several predictions about how environmental heterogeneity can influence the evolution of flowering strategy (Roff, 1992; Stearns, 1992). In the simplest case of annual vs. biennial flowering strategies, the optimal strategy depends on the lifetime fecundity of annuals and biennials, and the relative mortality of plants in the first year of growth relative to their mortality in the second year of growth (Charnov & Schaffer, 1973; Hart, 1977). Annuality is selectively favoured when mortality is low in the first year of growth and development is sufficiently rapid to enable high fecundity. In contrast, bienniality is favoured when mortality in the first year of growth is relatively high, mortality in the second year is low and delayed reproduction leads to greater fecundity than could be obtained after one year (Hart, 1977). This leads to the general prediction that high-quality environments with little competition (e.g. recently disturbed habitats) favour annual strategies (Hart, 1977; Law *et al.*, 1977), whereas the optimal strategy in lower quality environments varies according to how biotic and abiotic environmental factors affect age-specific mortality and fecundity. Studies have examined the influence of selection on specific traits correlated with variation in flowering strategy (Wesselingh & Klinkhamer, 1996; Donohue, 2002; Griffith *et al.*, 2004; Rose *et al.*, 2005), but research looking directly at the consequences of variable selection on flowering strategy itself (e.g. annual vs. biennial) is lacking.

In this study, I test the hypothesis that natural environmental variation causes  $G \times E$  in Common Evening Primrose (*Oenothera biennis* L., Onagraceae), which imposes variable selection on flowering strategy. *Oenothera biennis* is a native plant of North America that occurs in a variety of open habitats that vary in productivity. Although this species is typically described as a monocarpic biennial (Hall *et al.*, 1988), my preliminary observations indicated that *O. biennis* varied in annual and biennial strategies along productivity gradi-

ents. In this experiment, I utilized a natural environmental gradient to test three predictions: (i) variable selection along an environmental gradient causes  $G \times E$  for lifetime fitness of *O. biennis*; (ii) *O. biennis* exhibits genetic variation in flowering strategy on which selection can act; and (iii) genetic variation in plant fitness is correlated with variation in flowering strategy, so that  $G \times E$  imposes variable and potentially divergent selection on flowering strategy.

## Materials and methods

### Study site and system

This research was conducted in southern Ontario, Canada, at the Koffler Scientific Reserve at Jokers Hill (Jokers Hill). Jokers Hill is a 350-ha field station owned by the University of Toronto (<http://www.zoo.utoronto.ca/jokershill>), located 40 km N of Toronto, Ontario (44°03'N, 79°29'W, 300 m a.s.l.).

*Oenothera biennis* can live one (annuals), two (biennials) or three (triennials) years as a leafy rosette prior to flowering and death, although no triennials were observed in this experiment. Plants typically grow in open habitats where they form discrete patches of one individual to several hundred individuals. I define these patches of plants within single habitats as subpopulations, where populations are comprised of multiple subpopulations within separate habitats over a landscape. Clonal families (hereafter 'genotypes') of *O. biennis* collected from patches that are separated by hundreds of metres to several kilometres show quantitative genetic variation for many morphological, phenological and insect resistance traits (Johnson & Agrawal, 2005; McGuire & Johnson, 2006). Using allozyme markers, *O. biennis* shows little genetic variation within subpopulations, yet high genetic differentiation between subpopulations (Levin *et al.*, 1972; Levin, 1975). These patterns of genetic variation have been attributed to the functionally asexual behaviour of *O. biennis*; it has a permanent translocative heterozygote genetic system and primarily self-pollinates, which typically results in the production of clonally related seeds (Cleland, 1972). Because of this behaviour, numerous replicates of single genotypes can be grown from seed.

### Experimental design

A detailed description of the experiment is reported elsewhere (Johnson & Agrawal, 2005), so I only provide a brief description here. Seeds from 14 genotypes of *O. biennis* were collected between 2001 and 2002 from Jokers Hill (12 genotypes), Toronto (one genotype, 40 km S) and Mississauga (one genotype, 50 km SW). The 12 genotypes from Jokers Hill were collected from 11 discrete patches separated by 0.1–3.5 km. The genotypes from Toronto (genotype D) and Mississauga (K)

did not have extreme phenotypes (see Results) and were therefore included as was done in previous studies (Johnson & Agrawal, 2005; Johnson *et al.*, 2006; McGuire & Johnson, 2006).

Seeds were germinated in late-March 2002 and plants were transplanted into five common environments within natural habitats at Jokers Hill during May. The five habitats were separated by 0.5–3 km and they were selected to represent a productivity gradient (measured as mean dry aboveground biomass of *O. biennis*) that encompassed the range of environments in which *O. biennis* is locally found. These habitats included a dry, well-drained sloped field with low plant cover (Xeric), a recently mowed field with moderate plant cover (Mowed), a sloped field with sandy soil and moderate plant density (Sandy), a mesic old field with complete plant cover (Mesic) and a freshly ploughed field that was at first free from competing plants but was rapidly colonized by several plant species over the course of 2 years (Disturbed).

Habitats were divided into four equal-sized contiguous spatial blocks (hereafter microhabitats), and individual plants were planted into a grid planting design within microhabitats, with 1 m spacing between the rows and columns. In total, the experiment was comprised of 64–67 replicate plants per genotype for a total of 926 plants; plants from all genotypes were randomized and evenly replicated within each microhabitat and habitat. The aboveground portions of senescing flowering stalks (i.e. stems, stem leaves and fruits) were harvested in the last week of September in 2002 and 2003, whereas nonbolted rosettes were left intact. No plants survived beyond autumn 2003. Plants were dried at 60 °C and the dry biomass was measured from each plant. Total lifetime fitness was measured as the number of fruits produced by a plant from germination to plant death. A plant's relative fitness within a habitat was measured as the number of fruits produced by the plant divided by the mean number of fruits produced by all plants in a given habitat; the relative fitness of plants that produced no fruits was 0. I could not directly measure seed production because the fruits from the first flowers began to dehisce before plants had completed flowering. As *O. biennis* typically self-pollinates (Hoff, 1962; Ellstrand & Levin, 1982), the number of fruits reflects a measure of total male and female fitness.

Plants exhibited three flowering strategies: (i) plants that flowered in 2002 and subsequently died (annuals); (ii) plants that flowered in 2003 and then died (biennials); and (iii) plants that flowered in both 2002 and 2003 (iteroparous). Because iteroparity was only common in one habitat (Sandy), and iteroparous plants heavily weighted their reproductive effort to the second year of growth (see Results), I simplified flowering strategy into a dichotomous response (annual vs. biennial) based on the year in which the central stalk of the plant bolted. Statistical analyses would have been intractable other-

wise (Allison, 1999). I performed a separate analysis to explicitly test for genetic variation in iteroparity using the data from the Sandy habitat (see below).

## Statistical analyses

### *G × E and the maintenance of genetic variation*

I used mixed model analysis in PROC MIXED of SAS (SAS Inc., Cary, NC, USA; Littell *et al.*, 1996) to determine the effects of plant genotype and environment on plant fitness. The model for this analysis was: relative fitness = mean<sub>overall</sub> + habitat + genotype + microhabitat (habitat) + genotype × habitat + genotype × microhabitat (habitat) + error. I treated habitat as a fixed effect and determined its significance using weighted least-squares parameter estimation, with the degrees of freedom calculated by the Kenward–Roger method (Kenward & Roger, 1997); all other effects in the model were random. Restricted maximum likelihood (REML) was used to estimate the variance components of random effects, and their significance was calculated from log-likelihood ratio tests (Littell *et al.*, 1996, p. 44).

To assess whether  $G \times E$  can maintain multiple genotypes of *O. biennis* under soft selection, I followed the methods described by Stanton & Thiede (2005), who advocate the use of weighted analyses. In accordance with their methods, I used a modified Levene's test to identify the highest order interaction exhibiting heteroscedasticity (genotype × habitat). I then calculated precision from the residuals dataset for each level of the genotype-by-habitat interaction, and reanalysed the raw data, weighting each observation by precision using the WEIGHT statement in PROC MIXED. This procedure improved the fit of the model as measured by the Akaike information criterion. Results from analyses on the unweighted and weighted analyses are provided for comparison.

Genotype–environment interactions are most likely to maintain genetic variation when there is restricted gene flow among subpopulations (Christiansen, 1975; Maynard Smith, 1998) and the rank-order of genotypes changes among environments (Mitchell-Olds, 1992). Dispersal and the rate of migration between subpopulations has not been measured in *O. biennis*, but given the dehiscent nature of fruits and the lack of specialized dispersal structures, dispersal is likely to be very local and therefore migration between subpopulations should be infrequent (Willson & Traveset, 2000). A significant  $G \times E$  for relative fitness can be caused by either changes in the rank-order of mean genotype fitness among habitats (i.e. trade-offs in fitness) or differences in the magnitude of genetic variance among habitats. Only the former pattern can maintain genetic variation within populations. To measure the extent to which the rank-order in mean genotype fitness changed among habitats, I estimated the percentage of  $G \times E$  variance explained by crossing reaction norms among habitats (i.e. changes

in the rank-order of genotypes), compared to the percentage of  $G \times E$  variance explained by differences in the magnitude of genetic variance among habitats, using Cockerham's (1963, p. 88) equation:

$$\sigma_{\text{GEI}}^2 = \frac{\sum_{i=1}^h \sum_{j=1}^h [2\sigma_i\sigma_j(1 - r_{ij}) + (\sigma_i - \sigma_j)^2]}{h(h-1)},$$

where  $\sigma_i$  and  $\sigma_j$  are the square-root of genetic variance in the  $i$ th and  $j$ th habitat,  $r_{ij}$  the genetic correlation between habitats  $i$  and  $j$ , and  $h$  the number of habitats. The first half of the equation explains the variance due to crossing of reaction norms and the latter half represents changes in the magnitude of genetic variance between habitats. The genetic correlations among habitats were measured as Pearson correlation coefficients using the genotype best-linear unbiased predictors (BLUPs), and genetic variance was measured in each habitat using REML.

It is important to note that neither Stanton & Thiede's (2005) method nor the demonstration of substantial crossing of reaction norms using Cockerham's equation, provides a definitive test of whether  $G \times E$  can maintain multiple genotypes within a population. The presence of  $G \times E$  due to crossing reaction norms is a necessary condition for environmental variation to maintain genetic variation, but differences in the relative sizes of subpopulations coupled with substantial dispersal can counteract the effects that variable selection can have on the maintenance of genetic variation. A more complete knowledge of the relative sizes of natural subpopulations and typical dispersal rates would be needed to assess the role that environmental variation plays in the maintenance of genetic variation.

#### *Genetic variation for annual vs. biennial flowering strategy*

To examine how genotype and the environment affected whether a plant displayed an annual vs. a biennial strategy, I used generalized linear mixed models (GLMM; Wolfinger & O'Connell, 1993) in PROC GLIMMIX of SAS Version 9.1 (SAS Institute, Cary, NC, USA). GLMM is a pseudo-likelihood statistical procedure specifically designed to analyse mixed model data with categorical response variables (Wolfinger & O'Connell, 1993). In this analysis, I used the statistical model:  $N_{\text{flower2002}}/N_{\text{total}(2002+2003)} = \text{habitat} + \text{genotype} + \text{genotype} \times \text{habitat}$ , where  $N$  represents the number of individuals from a given genotype within a habitat. The data was fit to a binomial distribution with a logit-link function and I treated habitat as a fixed effect and the remaining factors as random; the significance of each effect was calculated as described above.

Because some genotypes were exclusively annual or biennial in certain environments, analyses had to be modified in two important ways. First, it was necessary to remove microhabitat from analyses. Second, all plants in the Disturbed habitat were annual, making analyses

intractable. To resolve this problem, I performed separate analyses with and without the Disturbed habitat data. Analyses without the Disturbed habitat were straightforward, but provide an incomplete picture of the data. To include all habitats in a single analysis, I transformed the data so there was at least one individual of each genotype that flowered in every habitat. This was accomplished by adding one biennial individual in cases where all replicates of a genotype in a habitat were annual, and the reverse for genotypes that had all replicates exhibiting a biennial strategy. For example, 15 of the 15 replicates of genotype N flowered during 2002 in the Disturbed habitat, which I transformed to 15 of 16 individuals. Simons & Johnston (2000) use a similar transformation to overcome the same statistical problem. This transformation makes the test for genetic and environmental effects conservative.

The use of GLMMs offers a powerful technique for testing the effects of genetic variation on traits that exhibit a discrete response. However, the theory and application of these methods are still under development (SAS Institute, pers. comm.) and the robustness of test statistics based on pseudo-likelihood statistics have not been demonstrated. Therefore, I also used generalized linear models in PROC GENMOD of SAS, which treats all factors as fixed effects and therefore offers a conservative test for significance of genotype and habitat  $\times$  genotype. Regardless of whether the Disturbed habitat is included or excluded, the main effect of genotype on flowering strategy remains significant ( $P < 0.001$ ), supporting the results from PROC GLIMMIX (see Results). In contrast with the results from PROC GLIMMIX (see Results), the genotype-by-habitat interaction becomes nonsignificant ( $P > 0.20$ ), which if true would indicate consistent effects of genetic variation in flowering strategy across all habitats. The mixed model analyses combined with the figures strongly suggest that the results from PROC GENMOD are too conservative and thus I only report the results from PROC GLIMMIX.

#### *Genetic variation for an iteroparous flowering strategy*

Although most plants were strictly either annual or biennial, many plants in the Sandy habitat were iteroparous, flowering in both 2002 and 2003. I examined whether there was genetic variation for iteroparity in the Sandy habitat by testing whether the frequency to flower in both years vs. in only a single year, was independent of plant genotype using a log-likelihood ratio  $\chi^2$ -test ( $G$ -test) (Sokal & Rohlf, 1995).

#### *Selection on annual vs. biennial flowering strategies*

I used analysis of variance (ANOVA) in PROC GLM of SAS to examine the phenotypic effects of flowering strategy on plant fitness. The statistical model for this analysis was: lifetime fruit production = mean<sub>overall</sub> + habitat + strategy + habitat  $\times$  strategy + error. No plants showed a biennial strategy in the Disturbed habitat so

this habitat was excluded from the analysis, and the effect of microhabitat was excluded because of unbalance in the data created by a paucity of annual plants in some spatial blocks. I conducted genotypic selection analyses using analysis of covariance (ANCOVA) to assess whether there was directional or stabilizing selection acting on flowering strategy (Rausher, 1992). For this analysis, habitat was treated as a categorical effect and the best-linear unbiased predictors (BLUPs) of genotype relative fitness were regressed against the proportion of annual plants within a genotype (annuality). I standardized annuality (i.e. the proportion of individuals within a genotype that were annual) within each habitat to a mean of zero and standard deviation of one (Lande & Arnold, 1983). There was no evidence of stabilizing or disruptive selection (i.e. the quadratic effect of annuality was not significant) and so only linear terms were included in the final model.

## Results

### Plant biomass and G × E effects on plant fitness

There was a gradient in productivity among habitats ( $F_{4,37} = 205.43$ ,  $P < 0.001$ ), whereby aboveground dry biomass of *O. biennis* varied by two orders of magnitude. The Xeric ( $2.79 \pm 0.32$  g, mean  $\pm$  SEM) and Mowed ( $7.53 \pm 0.69$  g) habitats had the lowest productivity, the Sandy ( $29.01 \pm 1.42$  g) and Mesic ( $43.71 \pm 2.25$  g) habitats were intermediate, and the Disturbed ( $277.40 \pm 8.54$  g) habitat had the highest productivity. This variation had important consequences for the effects of habitat on lifetime fruit production ( $F_{4,34} = 178.0$ ,  $P < 0.001$ ). As with plant biomass, absolute plant fitness was lowest in the Xeric ( $4.1 \pm 0.5$  fruits per plant) and Mowed ( $17.6 \pm 2.3$ ) habitats, intermediate in the Sandy ( $115.6 \pm 7.3$ ) and Mesic ( $138.1 \pm 6.9$ ) habitats, and greatest in the Disturbed habitat ( $961.6 \pm 28.4$ ).

Plant genotype and the environment interacted to affect relative fitness (Table 1). Several genotypes consistently exhibited higher than average relative fitness across habitats (Fig. 1). Relative fitness was also subject to a strong genotype-by-habitat interaction, where crossing of reaction norms explained 57 % of the total G × E variance, and 43 % of the variation was due to changes in genetic variance. Although several genotypes consistently had high relative fitness across habitats, no genotype had the highest relative fitness in more than two habitats (see Fig. 1). The effect of microhabitat was significant, indicating that small-scale environmental variation influenced relative fitness, but microhabitat and genotype did not interact (Table 1). There was no difference among habitats in relative fitness simply because relative fitness was calculated with respect to the mean fitness in each habitat.

**Table 1** The effects of habitat (H), plant genotype (G), microhabitat (M) and G × E [ $G \times H$  and  $G \times H(M)$ ] on relative fitness of *Oenothera biennis*.

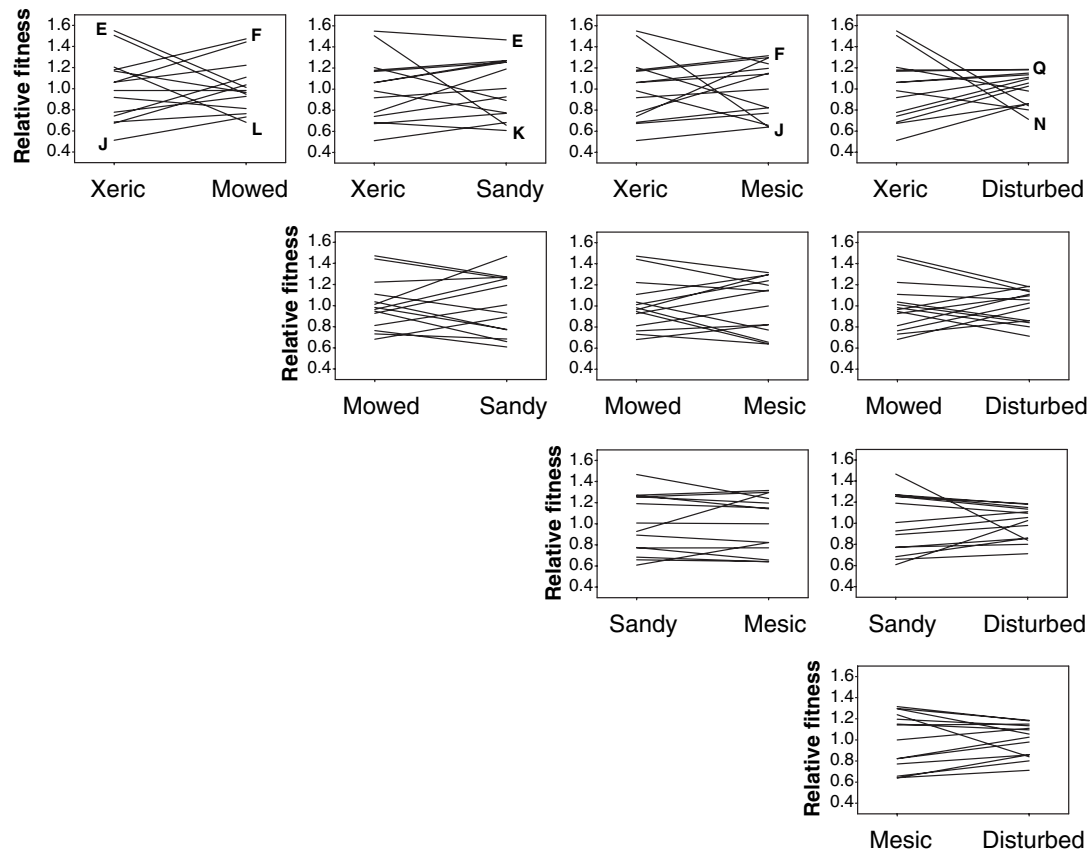
Source	Unweighted			Weighted		
	VC	$F/\chi^2$	<i>P</i>	VC	$F/\chi^2$	<i>P</i>
H	–	0	1	–	0.68	0.61
G	0.03	2.3	0.06	0.06	7.7	<b>0.003</b>
M(H)	0.08	16.4	<b>&lt;0.001</b>	0.02	27.5	<b>&lt;0.001</b>
$G \times H$	0.09	7.4	<b>0.003</b>	0.09	71.2	<b>&lt;0.001</b>
$G \times M(H)$	0.03	0.4	0.26	0.003	1.1	0.15
Residual	1.31			1.02		

Relative fitness was measured as the lifetime number of fruits produced by a plant, divided by the mean number of fruits produced by all plants in a particular habitat. The statistical significance of effects were determined using *F*-statistics for the fixed effect of habitat and  $\chi^2$  statistics from log-likelihood ratio tests for the random factors. The variance components (VC) for all random effects are reported. Unweighted analyses were performed on the raw data ('Unweighted' column), and analyses weighted by the precision statistic ('Weighted' column) (see Materials and methods). Effects significant at  $P < 0.05$  are in bold.

### Environmental and genetic effects on flowering strategy

Environmental variation had a prominent effect on flowering strategy. The percentage of plants that were annual in habitats with low (Xeric and Mowed) to medium productivity (Sandy and Mesic) ranged from 13 % and 20 % in the Xeric and Mowed sites, to 26 % and 24 % in the Sandy and Mesic sites respectively (Fig. 2). Despite this two-fold variation, the differences among these four habitats were only marginally significant (Table 2). In contrast, all plants were annual in the most productive habitat (Disturbed), resulting in a highly significant effect of habitat when Disturbed was included with the other four habitats in the analysis (Table 2). The variation in flowering strategy between habitats suggested that annuality is a phenotypically plastic trait of *O. biennis* that increases monotonically with habitat productivity.

*Oenothera biennis* also exhibited genetic variation in flowering strategy. The proportion of plants within a genotype exhibiting an annual behaviour varied from 23 % (genotype E) to 88 % (genotype N). The expression of this variation, however, depended on the environment, as indicated by the genotype-by-habitat interaction (Table 2). This interaction was partially due to the complete lack of variation within the disturbed habitat, contrasted against substantial genetic variation in all other habitats (Fig. 2). In the four habitats where there was variation, some genotypes were typically biennial (genotype E), others annual (genotype N), whereas several genotypes showed plasticity in annuality between all habitats (e.g. genotypes B and D).



**Fig. 1** Reaction-norm plots depicting  $G \times E$  for relative fitness of *Oenothera biennis*. Plant fitness of each genotype is relative to mean fitness within a habitat. Each panel contrasts fitness for each genotype between two different habitats, where each line depicts a unique genotype. The ends of each line are the best-linear unbiased predictors (BLUPs). The genotypes with the maximum and minimum absolute fitness in each habitat are shown in the top row of panels.

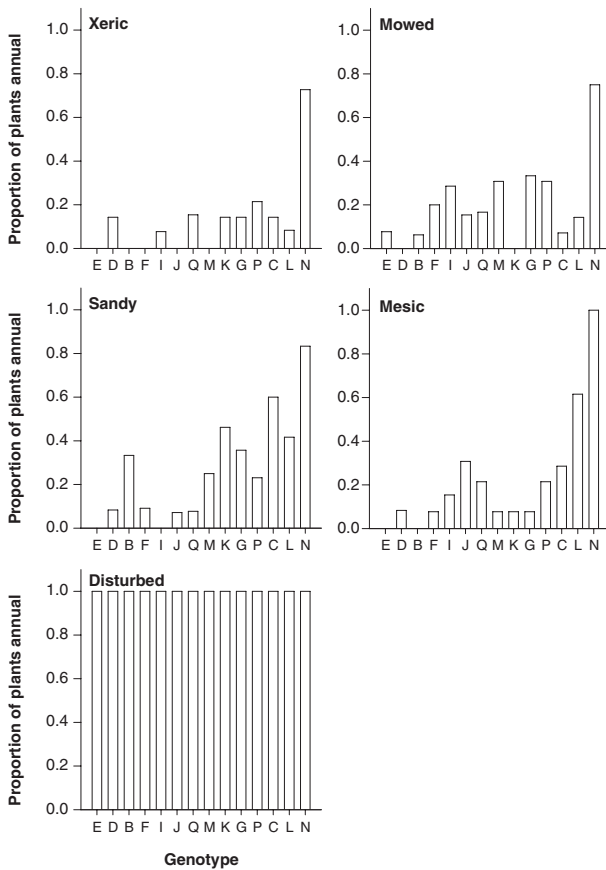
The change in the rank-order of genotypes for annuality also contributed to the significant genotype-by-habitat interaction. The effects of plant genotype and genotype-by-habitat interactions on flowering strategy were unchanged by the removal of the Disturbed habitat from analyses (Table 2).

Although I have treated flowering strategy as a dichotomy (annual vs. biennial) until this point, a third flowering strategy was common in the Sandy habitat, where 36 % of plants were iteroparous. Iteroparous plants flowered in both 2002 and 2003, and this iteroparity occurred when one to several axillary buds within the rosette bolted and flowered in 2002, whereas the apical meristem of the central stalk remained vegetative and flowered in 2003. The frequency of iteroparity varied from 0 % (genotype N) to 64 % (genotype I) between genotypes ( $G_{28} = 25.77$ ,  $P < 0.05$ ). Thus, there was genetic variation for iteroparity in the sandy habitat. Nearly all iteroparous individuals maximized reproductive effort in the second year (2002 fruits:  $14.7 \pm 2.7$ ; 2003 fruits:  $135.9 \pm 12.2$ ), and in this way they were functionally biennial.

### Selection on flowering strategy

Annual and biennial strategies varied substantially in lifetime fruit production. In phenotypic analyses (i.e. analyses that do not include plant genotype), the main effects of habitat ( $F_{3,716} = 79.43$ ,  $P < 0.001$ ) and flowering strategy ( $F_{1,716} = 68.46$ ,  $P < 0.001$ ) were both highly significant, and they interacted in a complex way to affect plant fitness (Strategy  $\times$  Habitat:  $F_{3,716} = 32.52$ ,  $P < 0.001$ ). In the low productivity Xeric and Mowed sites, annual plants had the highest lifetime fruit production (Fig. 3). In the medium productivity Sandy and Mesic sites, biennial plants had the highest lifetime fruit production. All plants in the Disturbed habitat were annual, which is also the habitat in which plants had the highest average lifetime fruit production (Fig. 3).

Consistent with the phenotypic selection analysis, the genotypic selection analysis detected variable selection on flowering strategy across habitats, and explained 36 % of the total variation in relative fitness. Overall, there was negative directional selection against annuality in favour of bienniality ( $F_{1,48} = 7.96$ ,  $P = 0.007$ ), but



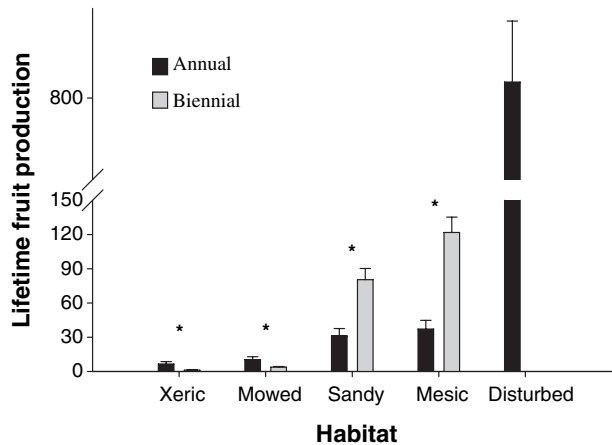
**Fig. 2** The effects of plant genotype and habitat on annuality in *Oenothera biennis*. Genotypes are ordered according to increasing experiment-wide proportion of annual plants.

**Table 2** Effects of habitat and genotype on flowering strategy.

Source	d.f.	$F/\chi^2$	<i>P</i>
(a) All habitats			
Habitat	4,63	44.28	<b>&lt;0.001</b>
Genotype	1	12.02	<b>&lt;0.001</b>
G × H	1	2.77	<b>0.048</b>
(b) Excluding disturbed			
Habitat	3,38	2.47	0.08
Genotype	1	17.56	<b>&lt;0.001</b>
G × H	1	2.75	<b>0.049</b>

Flowering strategy was treated as a dichotomous response, where a plant was either annual or biennial. The data was fit to a binomial distribution using pseudo-likelihood mixed model methods, which produced *F*-statistics for the fixed effect of habitat and  $\chi^2$ -statistics from log-likelihood ratio tests for the random factors (see Materials and methods). Effects significant at *P* < 0.05 are in bold.

habitat and strategy interacted ( $F_{3,48} = 4.52$ , *P* < 0.01) indicating that the strength of directional selection differed among habitats. Directional selection for an

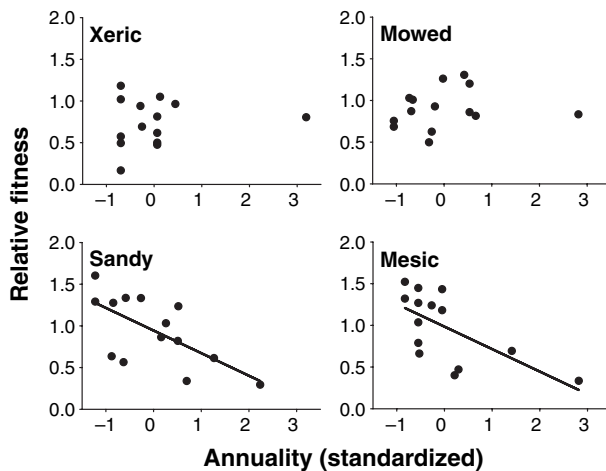


**Fig. 3** Lifetime fruit production and life-history strategy across five habitats. The habitats are arranged in increasing productivity from left to right. Annual plants (black bars) flowered in 2002 and biennial plants (grey bars) flowered in 2003. Bars depict the means plus one SEM from log-transformed data, back-transformed into the original units. Stars above bars indicate significant differences in fruit production between annual and biennial plants in each habitat according to the sequential Bonferroni correction (*P* < 0.001). No comparison was possible in the disturbed habitat because all plants were annual.

annual strategy was positive but nonsignificant in the Xeric ( $\beta = 0.03 \pm 0.08$ , *t* = 0.68, *P* = 0.68) and Mowed ( $\beta = 0.04 \pm 0.07$ , *t* = 0.55, *P* = 0.59) habitats, whereas selection on annuality was negative (i.e. selection for a biennial strategy) and significant in the Sandy ( $\beta = -0.27 \pm 0.09$ , *t* = -3.01, *P* < 0.01) and Mesic ( $\beta = -0.27 \pm 0.09$ , *t* = -2.94, *P* < 0.01) habitats (Fig. 4). The Disturbed habitat was not included in the analysis because there was no variation in flowering strategy.

**Discussion**

Three results from this experiment have important implications for the evolution of flowering strategy in *O. biennis*. First, plant genotype and habitat interacted to affect relative fitness, with the crossing of reaction norms explaining a substantial proportion of the G × E variance. Second, *O. biennis* genetically varied in whether it exhibited an annual or biennial flowering strategy. Third, genetic variation in fitness was correlated with genetic variation in flowering strategy, so that the G × E resulted in variable directional selection on flowering strategy. Specifically, annuals had higher or equal lifetime fitness than biennials in low productivity environments, whereas the reverse was true in moderately productive environments. As I discuss below, these results strongly suggest that natural environmental variation along a productivity gradient imposes divergent selection on flowering strategy in *O. biennis*.



**Fig. 4** Genotypic correlations depicting the selection gradients for relative fitness vs. annuality in the Xeric, Mowed, Sandy and Mesic habitats. Relative fitness was calculated as the best-linear unbiased predictor (BLUP) of a genotype's lifetime fruit production relative to the habitat mean from weighted analyses (see Materials and methods). Annuality was the proportion of plants that were annual within a genotype, standardized within each habitat to a mean of zero and SD of one. The regression lines depict significant selection gradients.

### Maintenance of genetic variation

Evolutionary theory predicts that  $G \times E$  can play a prominent role in maintaining genetic variation within populations (Levene, 1953; Dempster, 1955; Hedrick, 1986; Gillespie & Turelli, 1989; Byers, 2005). Analyses on relative fitness can be used to gain insight into the outcome of evolution in populations subject to soft selection (Stanton & Thiede, 2005). I found an overall effect of plant genotype on relative lifetime fitness, in addition to a  $G \times E$  caused by substantial crossing of reaction norms. This result suggests that  $G \times E$  may maintain genetic variation among subpopulations of *O. biennis*, however, a definitive test of this prediction requires information about the relative sizes of subpopulations and migration of genotypes between environments (Stratton & Bennington, 1998). Unfortunately, dispersal within *O. biennis* populations has not been measured, but given the dehiscent nature of fruits and the lack of specialized dispersal structures it can be inferred that seeds typically disperse at a very local scale (<2 m), with rare dispersal among subpopulations (Willson & Traveset, 2000). Such local dispersal is known to enhance the probability for  $G \times E$  to maintain genetic variation within populations (Christiansen, 1975). Thus, the  $G \times E$  observed in this experiment is likely sufficient to maintain genetic variation and provides a viable mechanism to explain the genetic differentiation observed among subpopulations and populations of *O. biennis* (Levin, 1975). This is not to say that  $G \times E$  is

the only mechanism, or even the most important mechanism operating within populations of *O. biennis*. Neutral processes (e.g. genetic drift, founder effects) and non-neutral mechanisms (e.g. mutation-selection balance) are likely to act in conjunction with  $G \times E$ .

### Genetic variation and variable selection on flowering strategy

Interactions due to  $G \times E$  can influence life-history evolution when genetic variation for components of fitness correlates with variation in life-history traits. I detected substantial genetic variation in flowering strategy of *O. biennis*, as there was variation for annual vs. biennial reproduction among genetic families in all but one habitat (Table 2, Fig. 2). This result provides evidence for local genetic variation in flowering strategy in a monocarpic plant, and builds on previous field studies that have detected genetic variation in flowering strategy among populations collected along broad latitudinal gradients (Reinartz, 1984; Lacey, 1988; Boudry *et al.*, 2002). The presence of such genetic variation at multiple spatial scales shows that monocarpic plant species often exhibit genetic variation within and between populations on which selection can act. I also observed genetic variation in semelparous vs. iteroparous reproduction in one habitat, and although there is a long history of theory on the evolution of these divergent strategies (Cole, 1954; Stearns, 1976), few studies have reported evidence for intraspecific genetic variation in these traits (Law *et al.*, 1977; Boudry *et al.*, 2002).

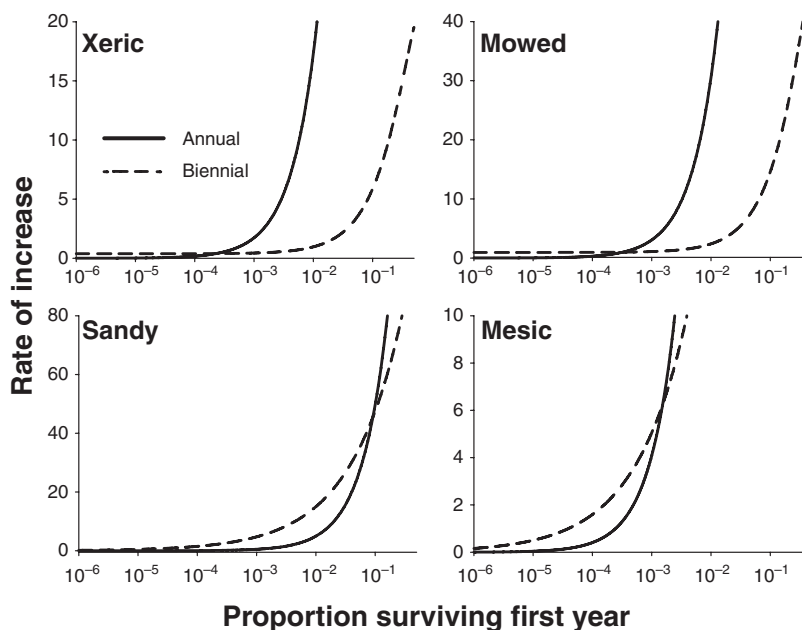
The  $G \times E$  affecting plant fitness resulted in variable selection on flowering strategy among the different habitats. The phenotypic effects of selection resulted in four-fold higher fruit production in annuals than biennials in the lowest productivity habitats, whereas fruit production of biennials averaged three-fold greater than annuals in the moderately productive habitats (Fig. 3). Genotypic selection analyses did not support the result from phenotypic selection analyses that annuals have higher fitness than biennials in low productivity environments (Fig. 4). Thus, the selective effects identified in phenotypic analyses were either due to an environmental correlation between annuality and fitness, or because of a lack of statistical power in the genetic correlations. In contrast, the genetic selection gradients in the Sandy and Mesic habitats support the conclusion that selection favours bienniality in moderately productive habitats (Fig. 4).

These empirical results parallel theoretical models on the evolution of biennial and annual strategies in monocarpic plant species. Models that incorporate local competition and the regulation of populations predict that the evolutionary stable strategy is the one that maximizes lifetime fecundity, irrespective of the length of the life cycle (de Jong *et al.*, 1987). My data clearly show that the lifetime fecundity of biennials is greater than that of annuals in the Mesic and Sandy habitats, whereas

annuality is likely favoured in low productivity sites and potentially high productivity sites as well (discussed below). However, lifetime fitness may not accurately portray the optimal strategy. For expanding populations in which local competition is weak, the optimal strategy is the one that maximizes the finite rate of increase ( $\lambda$ ) (Charnov & Schaffer, 1973). In such populations, the higher fecundity of biennials does not necessarily mean that this strategy is optimal, as the generation time of annuals is half that of biennials (Hart, 1977; Silvertown, 1983). In Hart's (1977) model,  $\lambda$  is influenced by three parameters: (i) the proportion of plants surviving from the seed stage to the end of the first growing season; (ii) the proportion of plants that survive to the end of subsequent seasons (given they survived the first year); and (iii) the number of seeds produced per plant. This model predicts that for *O. biennis*, annuality will be favoured in low productivity habitats over a broad range of parameter values for mortality during the first year of growth (Xeric and Mowed; Fig. 5a,b). In contrast, bienniality is the optimal strategy for *O. biennis* in the Mesic and Sandy habitats over a broad range of parameter values (Fig. 5c,d). Importantly, the range in first year survivorship over which bienniality is favoured corres-

ponds with estimated rates of survival in *O. biennis* (Gross & Werner, 1982; M. Johnson, unpublished data). The models considered here (Hart, 1977; de Jong *et al.*, 1987) encompass the plausible range of evolutionary scenarios expected to apply to life-history evolution in *O. biennis*. Both models support the conclusion that bienniality is optimal in moderately productive environments, whereas annuality will tend to be favoured in low productivity environments.

Is annuality favoured in high-quality environments? All plants were annual in the most productive environment (Disturbed) and they exhibited the highest absolute fitness across all habitats. Thus, the expression of an annual strategy appears to be highly canalized for *O. biennis* growing in high-quality habitats. Although there were no biennials for comparison, I speculate that selection favours an annual strategy in high-quality environments like the Disturbed habitat. These types of habitats are ephemeral, quickly becoming colonized by annuals and then perennials, and an annual strategy is therefore most likely to maximize fitness in a habitat prone to rapid succession. A similar result has been reported in a short-lived perennial grass (*Poa annua*), which evolved early reproduction and a short lifespan in



**Fig. 5** The rate of increase ( $\lambda$ ) for an annual and a biennial genotype in the Xeric, Mowed, Sandy and Mesic habitats as a function of first year survival. The data was adapted to Hart's (1977) models:  $\lambda_{\text{annual}} = C_1 S_a$  and  $\lambda_{\text{biennial}} = (C_1 C_2 S_b)^{0.5}$ , where  $C_1$  is the survival rate from seed to the end of the first growing season,  $C_2$  is the survival rate after the first season until reproduction,  $S_a$  and  $S_b$  are the number of seeds produced per annual and biennial plant respectively.  $C_2$  was 0.694 in Xeric, 0.804 in Mowed, 0.947 in Sandy and 0.971 in the Mesic habitats. The number of fruits produced by annual and biennial genotypes were estimated for each habitat from the genetic correlations of lifetime fruit production vs. annuality by extrapolating the equation of the line to the point where annuality equalled 1 and 0 respectively. The number of seeds produced by annual and biennial plants was then calculated as the number of fruits times the mean number of seeds produced per *Oenothera biennis* fruit ( $153.4 \pm 12.6$ ,  $n = 109$ ). The axis depicting the rate of increase ( $\lambda$ ) is rescaled in each panel to effectively illustrate the variation between an annual and a biennial strategy.

low competitive environments, and delayed reproduction and a longer lifespan in competitive environments (Law *et al.*, 1977).

### The evolution of flowering strategy in *Oenothera*

Species in the evening primrose genus (*Oenothera*) exhibit a diversity of life-history flowering strategies. Annual, biennial and iteroparous strategies are all found within the *Oenothera* genus, and in some clades these divergent strategies have evolved multiple times independently (Evans *et al.*, 2005). There are also anecdotal reports of mixed strategies within species (Klein, 1970; Dietrich, 1977; Hickman, 1993), although the genetic basis of such variation has not been previously studied. Among *Oenothera* spp., evolutionary transitions from perenniality to annuality are correlated with large-scale environmental gradients in aridity and precipitation (Evans *et al.*, 2005). Within a single *Oenothera* species, I found that environmental variation over relatively small spatial scales (0.5–3 km) can also lead to divergent selection on annual vs. biennial strategies in *O. biennis*, which provides insight into the microevolutionary processes that may have given rise to the diversity of life-history strategies across the *Oenothera* genus.

### Conclusions

Variable selection due to environmental heterogeneity has long been invoked as an important mechanism that maintains genetic variation within populations and drives the diversification of life-history strategies. I found that variable selection between habitats resulted in  $G \times E$  that affected lifetime fitness of *O. biennis* and variation in annual, biennial and iteroparous flowering strategies. My results show that  $G \times E$  imposes variable directional selection on genetic variation in flowering strategy, where annual and biennial strategies are favoured in different environments. Therefore, this study provides insight into the microevolutionary processes that underlie the maintenance of intraspecific variation in life-history traits within a native monocarpic plant.

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