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A shift towards the annual habit in selfing *Arabidopsis lyrata*

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An annual life history is often associated with the ability to self-fertilize. However, it is unknown whether the evolution of selfing commonly precedes the evolution of annuality, or *vice versa*. Using a 2-year common garden experiment, we asked if the evolution of selfing in the normally perennial *Arabidopsis lyrata* was accompanied by a shift towards the annual habit. Despite their very recent divergence from obligately outcrossing populations, selfing plants exhibited a 39% decrease in over-winter survival after the first year compared with outcrossing plants. Our data ruled out the most obvious underlying mechanism: differences in reproductive investment in the first year did not explain differences in survival. We conclude that transitions to selfing in perennial *A. lyrata* may be accompanied by a shift towards annuality, but drivers of the process require further investigation.

Subject Areas:
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1. Background

Mating system transitions from obligate outcrossing to predominant selfing are frequent evolutionary shifts in angiosperms [1]. Established selfing species display a characteristic flower morphology known as the 'selfing syndrome' [2,3] and are typically annual [4–6]. However, it is unclear whether this association exists because the transition to selfing is more likely in annuals, or because selection favours shorter lifespans in perennials that have evolved selfing. Most of our understanding about the evolutionary association between selfing and the annual life history is based on analyses that treat these as independent traits or assume that an annual or perennial life history is fixed and then explore the conditions favouring selfing. Empirical studies that consider the alternative, that shifts to selfing can select for changes in lifespan, are conspicuously lacking. To test if the transition to selfing in a perennial species can lead to a shift to annuality, we used the intraspecific mating system variation in North American populations of the ancestrally self-incompatible and perennial *Arabidopsis lyrata*, which has evolved several selfing populations. Specifically, we determined if survivorship in a native-range common garden is consistent with a shift towards a more annual life history in plants from recently evolved perennial selfing populations.

Several hypotheses could explain the link between lifespan and mating system, and the order in which these traits evolve. Selection could favour the evolution of selfing in annuals, as annuals have only one opportunity to reproduce and would incur a lower risk of reproductive failure if selfing evolved [7,8]. Alternatively, selection could favour the evolution of the annual habit in initially perennial selfing populations. The evolution of self-fertilization may increase the optimal level of total reproductive effort, thereby reducing survival and leading to an annual life history [9]. In particular, selfing in perennials could select for increased resource allocation to reproductive tissues rather than to vegetative structures needed for perennial survival, favouring the evolution of the annual habit [10]. The high costs of selfing in perennials due to inbreeding depression and/or seed discounting might also be ameliorated by a shift to annuality [11–13]. Furthermore, in selfing lineages, mutations that reduce lifespan could accumulate owing to neutral processes such as the build-up of drift load due to the lower effective population size associated with selfing [14,15]. Additionally, population bottlenecks before or during the formation of new selfing populations [16] may have fixed mutations that reduce lifespan. The roles of inbreeding depression and drift load in favouring the annual habit in perennial species that have transitioned to selfing can be evaluated by comparing crosses within and between populations that differ in their selfing rates. We are unaware of any experimental studies that have examined whether perennial species transition towards an annual habit after evolving to self.

Here, we used *Arabidopsis lyrata* to examine whether the evolution of selfing is followed by a transition towards the annual habit in newly diverged selfing lineages. This species is largely an obligate (self-incompatible) outcrosser but contains several self-compatible populations with high selfing rates [17]. These selfing populations likely originated from outcrossing ancestors less than 10 000 years ago, as they occur in previously glaciated habitat and lack a clear selfing syndrome [18]. Additionally, genetic evidence indicates selfing in these populations originated multiple times (electronic supplementary material, table S1) [17,19]. Studies both in the laboratory and/or under relatively mild environmental conditions have shown that both self-incompatible and selfing plants readily survive for multiple years [20,21]. Therefore, there is substantial evidence that all *A. lyrata* are ancestrally self-incompatible perennials and that novel selfing populations retain this perennial habit.

We monitored survival of progeny from crosses within and between selfing populations and crosses within and between outcrossing populations in a common garden within their native range over 2 years. Specifically, we asked: (1) whether plants from selfing populations had reduced over-winter survival compared with plants from outcrossing populations; and (2) whether between-population cross-progeny had higher survival than within-population cross-progeny (heterosis), and if this differed between selfing and outcrossing populations. Additionally, we asked (3) whether the selfed progeny of plants from selfing populations suffered from inbreeding depression. Furthermore, to test for a potential trade-off between survival and reproduction, we asked (4) whether plants that had a higher reproductive investment (i.e. flower and seed production) had lower survival.

2. Methods

(a) Study system

Arabidopsis lyrata subsp. *lyrata* (L.) is a perennial native to North American rock outcrops and sand dunes. Most populations are self-incompatible and highly outcrossing (multi-locus outcrossing rates $t_m > 0.8$; electronic supplementary material, table S1), with only a low frequency of self-compatible individuals [22]. However, self-compatibility has become fixed in some populations now characterized by high selfing rates [17,23] (t_m: 0.1–0.4; electronic supplementary material, table S1).

(b) Crossing designs

Our crossing designs are described in more detail elsewhere [24–26]. In brief, crosses involved plants (raised from field-collected seeds) from 12 *A. lyrata* populations with known mating systems: six populations of self-incompatible plants displaying high outcrossing rates (hereafter SI populations) and six populations of self-compatible plants displaying low outcrossing rates (hereafter SC populations) [17]. These populations likely represent multiple transitions to selfing [17,19]. We performed two consecutive rounds of crosses, each with different sets of parental plants. First, using five to eight plants per population, we performed within-population crosses to generate the following cross types: $\mathrm{SI} \times \mathrm{SI}_{\mathrm{within}}$, $\mathrm{SC} \times \mathrm{SC}_{\mathrm{within}}$, and SC × self. Second, for a further six plants per population, we performed within-population crosses (giving increased sample sizes for the $\mathrm{SI} \times \mathrm{SI}_{\mathrm{within}}$ and $\mathrm{SC} \times \mathrm{SC}_{\mathrm{within}}$ cross types) and between-population crosses (giving the two additional cross types $\mathrm{SI} \times \mathrm{SI}_{\mathrm{between}}$ and $\mathrm{SC} \times \mathrm{SC}_{\mathrm{between}}$ for testing heterotic effects, i.e. whether drift load affected survival). Crosses yielded 600 seed families in total.

(c) Common garden experiment

To evaluate whether the evolution of selfing is accompanied by a shift to the annual habit, we set up a common garden experiment at Trent University in Peterborough, Ontario, Canada. This location is at an intermediate latitude within the geographical range of the source populations (electronic supplementary material, table S1). From 20 to 22 March 2018, we sowed up to 50 seeds per seed family on peat-based substrate (one pot per family) in climate chambers (11 h days, 21/18°C day/night cycles, and 95% humidity). We planned to transplant three seedlings from each seed family, but three seedlings were not always available owing to insufficient germination of 279 families. When seedlings developed at least two true leaves (18 April–1 May), we transplanted one to three haphazardly chosen seedlings from each germinated seed family to individual pots, resulting in 915 experimental plants. On 10 May, prior to flowering, plants were moved to the common garden and organized in a randomized block design with three replicates of three 3 × 6 m blocks. Plants from each seed family and cross type were evenly distributed among the blocks.

(d) Winterization

On 9 November 2018, plants were winterized. To simulate natural winter conditions of snow cover that insulates meristems from deep-freezing temperatures, pots were packed closely together and then covered with a frost blanket and straw layer. A tarp was placed over the plants to hold the straw in place. On 7 April 2019, the plants were unpacked and returned to the original blocks.

(e) Survival and trade-offs with reproductive investment

To test whether plants from selfing populations showed lower survival to the second year than plants from outcrossing populations, we scored over-winter survival immediately after unpacking in 2019. Plants were considered to have survived when they had green living tissue or turgid roots. To test for a trade-off between survival and reproductive investment, we used the previous year's flowering data. For each plant, we had recorded whether it flowered and, if so, estimated how many flowers it produced throughout the season [24]. Additionally, to test for a trade-off between survival and reproductive output (i.e. first-year seed production), we estimated individuals' total seed production by multiplying fruit number by average seed number per fruit for each plant (electronic supplementary material, S2).

(f) Statistics

All statistics were performed in R 3.6.1 [27]. To test whether plants from selfing populations had lower survival than plants from outcrossing populations, and whether there were trade-offs between reproduction and survival, we used mixed-effects binary logistic regression models implemented in *lme4* [28]. These models included *plant survivorship* (response variable), *cross type* (fixed effect) and *maternal population* and *paternal population* (random effects). To test whether survival depended on flowering in the first year, we included *flowered in 2018* (binary) as a fixed effect. For the subset of plants that flowered, we tested whether survival depended on *flowering intensity* (estimated from the sum of all flowers produced in 2018; standardized using the *scale* function in R) included as a fixed effect. Additionally, to test whether survival depended on seed production in 2018, we included *total seed number* (standardized using the *scale* function in R) as a fixed effect. To assess the significance of the fixed terms, we re-fitted models using maximum likelihood and compared models with and without fixed terms using likelihood-ratio tests (LRTs [29]; table 1). For cross type, we defined a contrast matrix for *post hoc* comparisons with the *glht* function in the *multcomp* package (table 2) [30]. We calculated these contrasts for the model of survival including only *cross type* as a fixed effect because models that included additional fixed effects related to reproductive investment and their interactions with *cross type* were not significantly better at explaining survival (table 1). Specifically, we made four comparisons to test whether: (C1) crosses between populations resulted in higher survival than crosses within populations (heterosis); (C2) C1 depended on mating system; (C3) survival of progeny from self-compatible plants depended on whether they were produced by selfing or outcrossing (inbreeding depression); (C4) crosses among plants from selfing populations (SC × SC crosses) had lower survival than crosses among plants from outcrossing populations (SI × SI crosses).

3. Results

Cross type had a significant effect on over-winter survival. However, survival was not affected by any of our measures of first-year reproductive investment (table 1). Plants resulting from between-population crosses had a significantly higher survival than plants from within-population crosses (significant effect of C1; table 2 and figure 1). This effect did not significantly differ between SC and SI populations (no significant effect of C2; table 2). Within SC populations, progeny produced by selfing had similar survival to progeny produced by outcrossing (no significant effect of C3; table 2). Overall, plants from outcrossing populations (i.e. SI × SI crosses) had significantly higher over-winter survival than plants from selfing populations (i.e. SC × SC crosses) (45 versus 27%; significant effect of C4; table 2 and figure 1).

4. Discussion

Our key finding is that plants from selfing populations of *A. lyrata* had a 39% decreased over-winter survival rate when compared with plants from outcrossing populations, which was not explained by differences in first-year reproductive investment. Below we discuss the potential neutral and non-neutral drivers of this shift towards an annual life cycle.

Table 1. Mixed model analysis of over-winter survival of selfing and outcrossing *Arabidopsis lyrata*. Significance of fixed effects was evaluated using model comparisons with likelihood-ratio tests. Italic type indicates significant effects.

all experimental plants ($n = 915$)				subset of plants that flowered ($n = 549$)							
fixed effects	**d.f.**	**LRT**	**Pr(Chi)**	**fixed effects**	**d.f.**	**LRT**	**Pr(Chi)**	**fixed effects**	**d.f.**	**LRT**	**Pr(Chi)**
flowered 2018 (FL)	1	0.648	0.421	flowering intensity 2018 (FL_INT)	1	0.136	0.713	seed production 2018 (SP)	1	0.455	0.500
cross type (CT)	*4*	*21.307*	*<0.001*	cross type (CT)	*4*	*15.118*	*0.004*	cross type (CT)	*4*	*15.240*	*0.004*
FL : CT	4	1.193	0.879	FL_INT : CT	4	1.202	0.878	SP : CT	4	1.007	0.909
random effects	**d.f.**	**s.d. explained ($\text{d.f.}_{\text{residual}} = 903$)**		**random effects**	**d.f.**	**s.d. explained ($\text{d.f.}_{\text{residual}} = 537$)**		**random effects**	**d.f.**	**s.d. explained ($\text{d.f.}_{\text{residual}} = 537$)**	
maternal population	1	0.447		maternal population	1	0.366		maternal population	1	0.364	
paternal population	1	0.300		paternal population	1	0.258		paternal population	1	0.248	

Table 2. *Post hoc* hypothesis testing for survival using a contrast matrix (*glht* function in the *multcomp* package [30]). *z*, Wald statistic, SI, self-incompatible; SC, self-compatible. Significant effects are shown in italics.

					contrast definitions				
					outcrossing mating system		selfing mating system		
***post hoc* comparison**	**estimate**	**s.e.**	***z***	**Pr(>|z|)**	**$SI \times SI_{within}$**	**$SI \times SI_{between}$**	**SC × self**	**$SC \times SC_{within}$**	**$SC \times SC_{between}$**
C1, between- versus within-population crosses (heterosis overall)	*0.616*	*0.165*	*3.734*	*<0.001*	−1/2	1/2	0	−1/2	1/2
C2, interaction of mating system with C1	0.010	0.164	0.061	1.000	1/2	−1/2	0	−1/2	1/2
C3, difference between progeny produced by selfing and outcrossing (inbreeding depression)	0.146	0.348	0.418	0.988	0	0	−1	1	0
C4, difference between mating system (SI versus SC)	*0.913*	*0.349*	*2.617*	*0.035*	1/2	1/2	−1/3	−1/3	−1/3

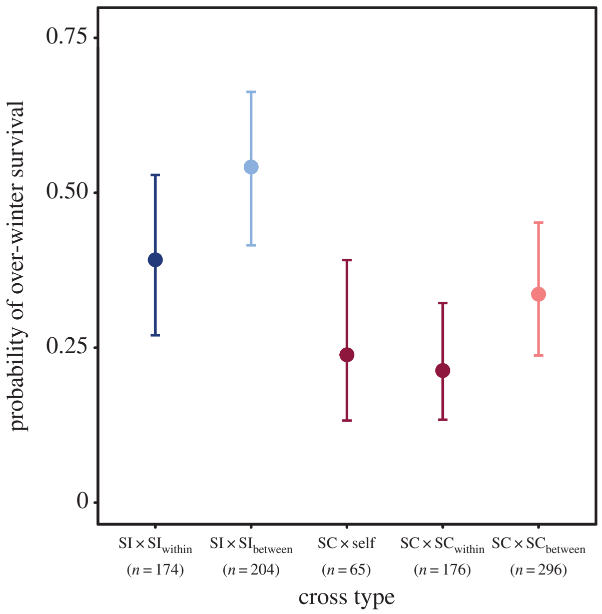

Figure 1. Effect plot of over-winter survival by cross type, $n = 915$. Predictions from the binary logistic regression model were obtained with the *ggpredict* function in the *ggeffects* R package [31]. Coloured points are predicted values and error bars represent 95% confidence intervals. The between-population cross types ($SI \times SI_{between}$ and $SC \times SC_{between}$) had significantly higher survival than their respective within-population cross types ($p < 0.001$). The outcrossing mating system (represented by the cross types $SI \times SI_{within}$ and $SI \times SI_{between}$) had significantly higher survival than the selfing mating system (represented by cross types SC × self, $SC \times SC_{within}$ and $SC \times SC_{between}$) ($p = 0.035$).

Neutral processes could be influencing the genetic background of selfers and facilitating selection towards a more annual habit. Selfing populations often experience genetic degradation through processes such as inbreeding depression and the accumulation of drift load. Although inbreeding depression has been identified for *A. lyrata* vegetative biomass [25,26], SC × self and $SC \times SC_{within}$ crosses did not differ in survival. Therefore, our results are not consistent with inbreeding depression affecting over-winter survival. It is also likely that *A. lyrata* populations have gone through repeated cycles of colonization and extinction [15,32] and thus locally contain an excess of deleterious alleles [33]. In line with this, we found that between-population crosses resulted in significant heterosis in survival. This heterosis was of similar magnitude for SI and SC populations, and thus did not restore SC plant survival to the levels observed in crosses between SI plants. This suggests that SI and SC populations harbour similar levels of drift load. While our results agree with previous findings that drift processes are important in *A. lyrata* [20], drift alone seems unlikely to explain the observed differences in over-winter survival between selfing and outcrossing populations.

Among the potential non-neutral drivers, trade-offs between reproduction and meristems needed for over-winter survival are often presumed to be a critical factor in driving transitions to annuality [10]. However, our data are not consistent with reproductive trade-offs explaining a shift towards annuality in *A. lyrata* selfing populations. Plants that flowered in the first year were as likely to survive over-wintering as plants that postponed flowering. Also, survival did not depend on flowering intensity or seed production. Yet, there could be a genetic correlation between floral traits and lifespan, so that selection for increased pollen : ovule ratios [18] indirectly selected for reduced lifespan. However, this would not explain why previous work in non-native, more-temperate environments found no differences in over-winter survival between selfing and outcrossing *A. lyrata* [20,21]. Furthermore, greenhouse experiments with common agricultural pests have shown that SC plants are not more susceptible to infection or herbivory than SI plants [34,35]. Therefore, we suggest that environmental conditions specific to our experiment led to the reduced lifespan in selfers. These conditions could include biotic factors such as native herbivores or pathogens, and/or abiotic factors such as the extreme fluctuations in temperature that characterize the continental climate of southeastern Canada. How environmental factors interact with the genetic consequences of selfing to cause the reduced over-winter survival of selfers remains to be investigated.

Data accessibility. The data associated with this manuscript are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.ht76hdrck. [36]

Authors' contributions. C.G. and M.S. conceived the study. C.G., M.v.K., M.E.D. and M.S. designed the experiment. C.G. and C.S. collected the data. C.G. and M.S. analysed the data with input from M.v.K. and M.E.D. C.G. and M.S. wrote the paper with input from all authors. All authors have approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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