

Reproductive ecology of the distylous species *Houstonia longifolia*: implications for conservation of a rare species

Jennine L.M. Pedersen, S. Ellen Macdonald, and Scott E. Nielsen

Abstract: Distylous species typically experience self-incompatibility with one morph often having partial self-compatibility. Small populations may therefore experience greater rates of selfing/intramorph crosses leading to skewed morph ratios and reduced seed production. For the distylous species *Houstonia longifolia* Gaertn. (“imperiled” at its northwestern range limit in Alberta), we examined whether small populations were morph-biased and whether seed production was affected by population size, local density, plant size, morph type, and surrounding morph ratio. For focal plants in several populations, we measured size (height, number of stems) and local density (1 m²) of pins and thrums, with the focal plants collected for seed counts. Population size was estimated from densities in systematically located quadrats in each population. Morph ratios were pin-biased in small populations but were even to slightly thrum-biased in large populations. The critical population size for maintaining an equal morph ratio was ~726 plants. Seed production was most influenced by the interaction between morph type and surrounding morph ratio, which were themselves influenced by population size (Allee effect). Seed production increased for thrums but decreased for pins as the proportion of surrounding pins increased, suggesting strong incompatibility. These results provide guidance on population size and morph ratios for conservation actions.

Key words: distylous, self-incompatibility, morph ratio, seed production, Allee effect.

Résumé : Les espèces distyles sont typiquement auto-incompatibles, un des morphes présentant souvent une auto-compatibilité partielle. De petites populations peuvent ainsi présenter des taux d'autofécondation ou de croisement intra-morphes plus élevés, résultant en des ratios faussés de morphes et en une production réduite de semences. En ce qui concerne l'espèce distyle *Houstonia longifolia* Gaertn. (menacée dans sa limite de distribution au nord-ouest de l'Alberta), les auteurs ont examiné si de petites populations présentaient un biais quant aux morphes et si la production des semences était affecté par : la taille de la population, la densité locale, la taille de la plante, le type de morphe et le ratio environnant de morphes. Ils ont mesuré chez les plantes focales de plusieurs populations la taille (hauteur, nombre de tiges) et la densité locale (1 m²) de longistyles et brévistyles, les plantes focales étant récoltées aux fins de décompte de semences. La taille de la population était estimée à partir des densités des quadrats systématiquement situés dans chaque population. Les ratios de morphes étaient biaisés en faveur des longistyles dans les petites populations, mais même légèrement biaisés en faveur des brévistyles dans les grandes populations. La taille critique de la population afin de maintenir un ratio égal de morphes était d'environ 726 plants. La production de semences était surtout influencée par l'interaction entre le type de morphe et le ratio environnant de morphes, qui étaient eux-mêmes influencés par la taille de la population (effet Allee). La production de semences augmentait chez les brévistyles mais diminuait chez les longistyles à mesure de l'augmentation de la proportion de longistyles environnants, suggérant une forte incompatibilité. Ces résultats donnent une orientation quant à la taille de la population et le ratio des morphes à maintenir en vue d'actions de conservation. [Traduit par la Rédaction]

Mots-clés : distyle, auto-incompatibilité, ratio de morphes, production de semences, effet Allee.

Introduction

Heterostyly is a type of sexual polymorphism found in 28 plant families in which plants produce either two (distyly) or three (tristyly) floral morphs (Barrett 2002). Distylous species exhibit herkogamy within flowers, with pins having long styles and short stamens, and thrums

having short styles and long stamens (Ganders 1979; Barrett 2002). Thus, in pin plants, the stigma is positioned above the anthers, whereas the reverse is true in thrum plants. Most distylous plants express heteromorphic incompatibility; only crosses between pins and thrums lead to fertilization (Ganders 1979; Barrett 2002).

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This incompatibility system is believed to be maintained by the spatial separation of sexual structures in flowers (Barrett 2002), promoting pollen transfer from short stamens to short pistils and long stamens to long pistils (Ganders 1979; Meeus et al. 2012).

In most distylous species, pins are recessive homozygous (ss) and thrums are dominant heterozygous (Ss) (Lewis and Jones 1992). This incompatibility system is assumed to result in equal proportions of pin and thrum offspring (i.e., seed), thus maintaining equal morph ratios (Van Rossum et al. 2006; Meeus et al. 2012). However, some distylous species may experience a breakdown of self-incompatibility (Ganders 1979; Barrett 1989, 2013). This breakdown has been shown to occur more frequently for pin morphs (Ganders 1979), although this can vary even within the same family (e.g., Rubiaceae family; Bahadur 1970a; Wyatt and Hellwig 1979). These breakdowns are believed to increase selfing and intramorph crosses in those populations experiencing intermorph disruptions leading to morph biases toward the more self-compatible morph (Ganders 1979; Van Rossum et al. 2006; Meeus et al. 2012). Small populations are more likely to experience these disruptions because they are prone to “demographic stochasticity and genetic drift” (Van Rossum et al. 2006), resulting in a lack of compatible mates (Endels et al. 2002; Kery et al. 2003; Brys et al. 2008). Large populations are therefore expected to have balanced morph ratios and higher pollination levels compared with small populations; in turn, this will result in higher levels of intermorph crosses (Kery et al. 2000; Matsumura and Washitani 2000; Jacquemyn et al. 2002; Kery et al. 2003). Further, this will lead to higher seed production because intermorph crosses result in higher seed sets than intramorph crosses (Beliveau and Wyatt 1999; Shibayama and Kadono 2003; Van Rossum et al. 2006). Thus, it is believed that distylous species experience a component Allee effect in which seed production is influenced by the morph ratio in varying population sizes through a mechanism of morph-biased reproductive incompatibility similar to the mechanism of “sex ratio fluctuations” as described by Stephens et al. (1999).

Seed production has been shown to differ among floral morphs (Ågren and Ericson 1996; Beliveau and Wyatt 1999; Matsumura and Washitani 2000; Minuto et al. 2014) and vary with plant size. Larger plants produce more flowers and experience higher pollinator visitation rates (Schmitt 1983; Brothers and Atwell 2014). Likewise, local floral density affects seed production (Feinsinger et al. 1991) with smaller distances between morph types increasing the rate of intermorph crosses (Nicholls 1987; Shibayama and Kadono 2003). Past studies have demonstrated seed set to be positively related to the frequency of pins in a population (Van Rossum et al. 2006; Brys et al. 2008). This is likely due to thrums requiring pins for intermorph crosses, or perhaps pins being more self-

compatible, allowing for seed production even in the absence of thrums (Ganders 1979). However, to our knowledge, no studies have investigated how the number of pins in close proximity to a focal plant of varying morph type affects seed production.

For this study, the reproductive success of the distylous species long-leaved bluet (*Houstonia longifolia* Gaertn.; Moss 1983) was investigated. Factors examined included population size, local density, proportion of pins around focal plants, morph type (pin or thrum), and plant size (maximum height and number of stems). Our objectives were to (i) determine whether small populations had morph ratios biased toward the pin morph; this was predicted to be the more self-compatible morph since breakdowns in self-incompatibility occur more frequently for this morph type (Ganders 1979); and (ii) examine which factors most influence this species' seed production by testing three hypotheses: component Allee effect, local density, and incompatibility. For the component Allee effect hypothesis, we predicted that seed production would be positively related to population size and (or) even morph ratios. For the local density hypothesis, we predicted that increasing local density of *H. longifolia* would increase seed production of a focal plant regardless of the morph ratio. For the incompatibility hypothesis, we predicted that seed production of a focal plant would increase with increases in the proportion of the opposite morph in surrounding plants because this would indicate this species has an incompatibility system common for distylous species. Plant size was incorporated in all hypotheses tested because taller multistemmed plants are likely to produce more seed.

Materials and methods

Study species

The long-leaved bluet (*H. longifolia*) in the subgenus *Chamisme* of Terrell (1991, 2007) is a distylous perennial forb within the Madder family (Rubiaceae). This species has opposite leaves and purplish to white corollas; each plant consists of multiple (1–100) short (~15 cm) reproductive stems containing multiple flowers in a cyme arrangement (Beliveau and Wyatt 1999; Royer and Dickinson 2007). *Houstonia longifolia* is found in five Canadian provinces (Alberta, Saskatchewan, Manitoba, Ontario, and Quebec) and across the midwestern and eastern USA, having a global ranking of G4G5 (NatureServe 2013). The northwestern range limit of the species is in central Alberta, where its conservation status is imperiled (S2) (NatureServe 2013) and its distribution is restricted to the Central Parkland Natural Subregion (Alberta Conservation Information Management System [ACIMS] 2015). This species is found on sandy woodlands and dunes (Royer and Dickinson 2007), and also along sandy roadside ditches (J.L.M. Pedersen, personal observation, 2014). In Alberta, it flowers from early June through July (Beliveau and Wyatt 1999) with its seeds maturing by September

(J.L.M. Pedersen, personal observation, 2014). Based on one day of observation (16 June 2015), floral visitors of this species include Eastern flower thrips (Thysanoptera: Thripidae: *Frankliniella tritici* (Fitch)), flea beetles (Coleoptera: Chrysomelidae: *Altica* sp.), hover flies (Diptera: Syrphidae: *Paragus haemorrhous* (Miegen), *Sphaerophoria philanthus* (Miegen)), bee flies (Diptera: Bombyliidae: *Hemipenthes*, 2 spp.), digger wasps (Hymenoptera: Sphecidae: *Ammophila* sp.), bees (Hymenoptera: Apidae: *Nomada* sp., Megachilidae: *Osmia*, 2 spp., *Hoplitis pilosifrons* (Cresson), *Dianthidium* sp., Halictidae: *Lasioglossum*, 3 spp.), and ants (Hymenoptera: Formicidae: *Formica podzolica* Francoeur). This species has ballistic dispersal, yet is considered to be dispersal limited because it lacks any seed features designed to improve dispersal (Kershaw et al. 2001) and has small seeds (estimated in our populations to be 11 714 seeds per gram; SE = 969; $n = 5$ plants, each at 500 seeds).

Study area

The study was conducted within the Central Parkland Natural Subregion in Alberta, which has a mean annual temperature of 2.3 °C, a mean annual precipitation of 441 mm, and 1412 growing degree days above 5 °C (Natural Regions Committee [NRC] 2006). A mixture of deciduous forests and grasslands characterize this area. This region is also considered to have some of the highest levels of habitat fragmentation and habitat loss in Alberta, owing to oil and gas exploration and exploitation, grazing, and agriculture (NRC 2006). *Houstonia longifolia* populations found within this area were separated by areas of unsuitable habitat, such as forests, wetlands, roadways, and especially cultivated agricultural land.

Focal plant surveys

Prior to commencement of field work, preliminary investigations were completed including review of the phenological characteristics of *H. longifolia*, review of the Alberta Conservation Information Management System (ACIMS) occurrence records, consultations with Alberta Native Plant Council (ANPC) members, and searches of suitable sandy habitat using aerial imagery from Google Earth. From this, a list of sites was generated for survey visits. Meander searches were then used at each site to identify locations with *H. longifolia* and to determine the extent of detected populations (following rare plant survey methods described in Lancaster 2000; Henderson 2009). Targeted surveys were then undertaken at these sites from late June to mid-July 2014 during the *H. longifolia* flowering period. From these surveys, a total of 14 populations of *H. longifolia* were found within the Central Parkland Natural Subregion (Table 1). Following these targeted surveys, meander surveys were conducted at each location to locate focal plants. Depending on population size, between 4 and 26 individuals (focal plants) were marked at each of the 14 populations for a total of 210 *H. longifolia* plants (Table 1). Focal plants were chosen based on different densities of *H. longifolia* plants sur-

rounding them with multiple plants of each morph type being included.

Plants were marked at each location using a flag with an identification number given to indicate site location and morph type. A nail was also placed at the base of each plant and marked with its floral morph as a secondary marker. Although identification of pin and thrum floral morphs could be determined by the naked eye when examined closely, magnifying lenses were also used. For each marked plant, global positioning system (GPS) coordinates were recorded along with the maximum and mean (based on 10 stems) stem height.

Local density and population size

A circular quadrat 1 m² was placed around each focal plant and used to quantify local density and the number and proportion of pins and thrums surrounding each focal plant. Some plants surrounding the focal plant lost their flowers before morph identification could occur. These individuals were recorded as unknown, but were still used in calculating local density for each focal plant.

To determine population size, we conducted transect searches (100 m parallel transects bisecting populations) in July 2014 in each of the 14 populations following survey methods from Henderson (2009). Search intensity at each location varied with the size of the population and visibility of *H. longifolia* (as recommended by Lancaster 2000 and Henderson 2009 for rare plant surveys). For each transect, a 1 m² circular quadrat was systematically spaced every 2 to 5 m, depending on the terrain and extent of population. In each quadrat, the number of individuals present was recorded. Since plants were clumped spatially, individual counts were based on number of rosettes of basal leaves. Distances between transects varied from 3 to 20 m, again based on terrain and extent of population (Table 1). Only one survey occurred for each population.

Following each survey, extents of populations were defined using the track function on a GPS (Garmin Oregon 550) and walking the boundary of populations or, in some cases, using the start and end points of transects to define extents of populations. From these extents, total area (m²) of each population was calculated using ArcGIS 10.2.

Total number of individuals in all 1 m² circular quadrats was divided by the total number of quadrats to estimate mean *H. longifolia* density for each population. This mean density (per 1 m²) was multiplied by the areal extent (total area m²) of each population to estimate total *H. longifolia* population size (Table 1). The standard error for mean density per transect was used to estimate the 95% confidence interval for population size (SE × 1.96).

Seed production

In September 2014, all 14 populations were revisited and the 210 focal plants were collected just prior to seed dispersal and full senescence of plants. The capsules of these plants were brittle at the time of collection. Therefore,

Table 1. Summary of *Houstonia longifolia* populations included in the study and details of sampling and plant collection for each.

Locations	Latitude (GPS)	Longitude (GPS)	No. of transects	Spacing between transects (m)	Mean density (no. plants/m ²), mean (SE)	Total area (m ²)	Population estimate (95% CI)	No. focal plants collected	Morph ratio (95% CI)
Gibbons Field (private property)	Confidential		20	10	1.23 (0.45)	40 000.0	49 200 (±35 220)	20	-0.12 (±0.31)
Gibbons Dune	53.863	-113.319	9	15	0.55 (0.46)	10 000.0	5500 (±8979)	23	-0.21 (±0.29)
North Bruderheim Provincial Recreation Area	53.856	-112.934	82	20	0.02 (0.03)	200 180.5	4004 (±3652)	26	-0.15 (±0.27)
West Bruderheim	53.865	-112.928	18	5 (small areas) or 10 (large areas)	0.16 (0.11)	19 514.1	3122 (±4313)	23	-0.13 (±0.29)
Northwest of Bruderheim Natural Area	53.850	-113.029	25	4	0.12 (0.07)	13 327.2	1599 (±1859)	23	0.19 (±0.29)
East Bruderheim	53.867	-112.923	27	5 (small areas) or 10 (large areas)	0.06 (0.03)	16 809.9	1009 (±954)	13	0.01 (±0.39)
Pipeline clearing near Andrew, AB	53.981	-112.759	20	5	0.07 (0.02)	11 800.0	826 (±513)	9	-0.04 (±0.46)
Lily Lake Natural Area	53.949	-113.372	7	4	0.16 (0.12)	3746.0	599 (±905)	4	-0.02 (±0.69)
Fort Saskatchewan Prairie	53.681	-113.270	20	15	0.02 (0.01)	27 193.0	544 (±777)	12	0.02 (±0.40)
Bellis Lake Natural Area	54.115	-112.173	52	5 (small areas) or 10 (large areas)	0.02 (0.00)	26 983.0	540 (±426)	25	-0.23 (±0.28)
Ditches near Smokey Lake	54.046	-112.357	6	3	0.19 (0.12)	1145.6	218 (±278)	14	-0.11 (±0.37)
Property near Bruderheim	Confidential		3	3	0.45 (0.36)	162.8	73 (±114)	6	0.45 (±0.56)
Berm of Wellsite	53.833	-113.052	6	3	0.02 (0.01)	1121.0	22 (±20)	7	0.20 (±0.62)
Railway Park	53.811	-113.051	5	3	0.08 (0.04)	272.3	22 (±23)	5	0.48 (±0.52)

Note: West Bruderheim refers to northwest of Bruderheim Provincial Recreation Area on the west side of the Range Rd 204, whereas East Bruderheim refers to this recreational area on the east side of the road. Spacing between transects was 5 m for Gibbons Field and Gibbons Dune and 2 m for all other locations. Population size estimates (95% CI) were calculated by multiplying mean density (no. plants per m²) by total habitat area (m²). Morph ratio values, calculated as pin - thrum/(pin + thrum), range from -1 (all thrums) to +1 (all pins) with zero indicating equal proportions of the two morphs.

Table 2. Hypotheses (candidate models) for predicting seed production in *Houstonia longifolia*.

Hypothesis	Model	Predictions
Ecological base	L+S+H	Plant size (maximum height and no. of stems) predicted to be positively related to seed production
Allee (population size)	L+S+H+N	Population size predicted to be positively related to seed production
Density (local density)	L+S+H+D	Local density predicted to be positively related to seed production
Allee and density	L+S+H+N+D	Population size and local density predicted to be positively related to seed production
Incompatibility	L+S+H+M×P	Incompatibility system predicted to influence seed production (i.e., seed production of a focal thrum plant predicted to be positively related to proportion of pin plants in the surrounding area and in focal pin plants to be negatively related to proportion of pins); this effect could be stronger for thrum than for pin plants
Allee and incompatibility	L+S+H+N+M×P	Incompatibility system (as above) and population size predicted to be positively related to seed production
Density and incompatibility	L+S+H+D+M×P	Incompatibility system (as above) and local density predicted to be positively related to seed production
Allee, density, and incompatibility	L+S+H+N+D+M×P	Incompatibility system (as above) and both population size and local density predicted to be positively related to seed production

Note: L, location (random); H, maximum plant height; S, no. of stems; M, morph; N, population size; P, proportion of pin; D, density (within 1 m² quadrat surrounding focal plants).

to prevent the loss of seeds, full stems were collected. During collection, coin envelopes were placed over each plant, the stems were cut at the base near the basal leaves, and the loose stems were then tipped upside down into the labeled coin envelope. Owing to the small size of seeds (<1 mm), envelopes had their flaps taped and were placed into plastic bags to prevent seed loss during transport. Plastic bags were removed in the lab, and any seeds contained in these bags were transferred to a new coin envelope labeled with the same ID. The coin envelopes were then placed into brown paper bags and left in a cool dry place to promote further drying and seed ripening.

Owing to their small size, seeds for each marked plant were manually counted. For each plant, the number of stems was counted (mean number of stems = 17) and any unopened capsules containing seeds were opened with tweezers. Magnifying lamps were used to assist with seed counting. To ensure the accuracy of seed counts, a sample of plants (6%) were double-counted with a mean error rate estimated at 4.0% (see Supplementary data for recount data of *Houstonia longifolia* seeds)¹.

Data analyses

The statistical software package R 3.1.2 was used to conduct all analyses (R Core Team 2014). The largest population (~49 200 individuals over 40 000 m²) sampled was removed from analyses because its population size was an order of magnitude larger than any other population (next largest was ~5500); with no observations of intermediate population sizes, this resulted in a single large outlier in the dataset (see Supplementary data for analysis including the largest population)¹. Thus, we

used 190 of the 210 collected focal plants from a total of 13 populations that ranged from ~22 to ~5500 individuals of *H. longifolia*.

Morph ratio was calculated as: (no. of pins – no. of thrums)/(no. of pins + no. of thrums) (Meeus et al. 2012). A morph ratio of –1.0 indicates populations of only thrums and +1.0 indicates populations of only pins, with zero indicating an equal morph ratio (Meeus et al. 2012). A linear-log regression model using the statistical software package R 3.1.2 was used to determine the effect of population size (log₁₀ transformed) on morph ratio and estimate the population size at which the morph ratio was equal (zero).

We compared eight candidate models (negative binomial generalized linear mixed effects) to test our hypotheses regarding the factors affecting seed production in *H. longifolia* (Table 2). Data from the 190 focal plants were used for model construction. Model 1 was an ecological base model in which seed production (no. of seeds per plant) was the response variable, location (i.e., population) was a random factor, and measures of plant size (no. of stems and maximum height) were included as covariates because size is likely related to seed production. The three models to test the Allee, density, and incompatibility hypotheses were constructed by inclusion of population size, local density, or the interaction between morph type and morph ratio (as indicated by proportion pin), respectively, into the ecological base model (Table 2). The interaction between morph type and proportion pin was included for the incompatibility hypothesis model to determine if seed production would vary with morph type of the focal plant and the propor-

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2016-0024>.

tion of morph type (i.e., pins) surrounding it. Four combined models were then produced by incorporating the predictor variables from the Allee, density, and incompatibility hypothesis models in various combinations as follows: an Allee and density hypothesis, including population size and local density; an Allee and incompatibility hypothesis, including population size, morph type, and proportion pin; a density and incompatibility hypothesis, including local density, morph type, and proportion pin; and an Allee, density, and incompatibility hypothesis that incorporated all predictor variables (Table 2). For these eight models, all continuous fixed predictor variables including covariates were log transformed to improve model convergence, except for the variable proportion pin, which was arcsine transformed.

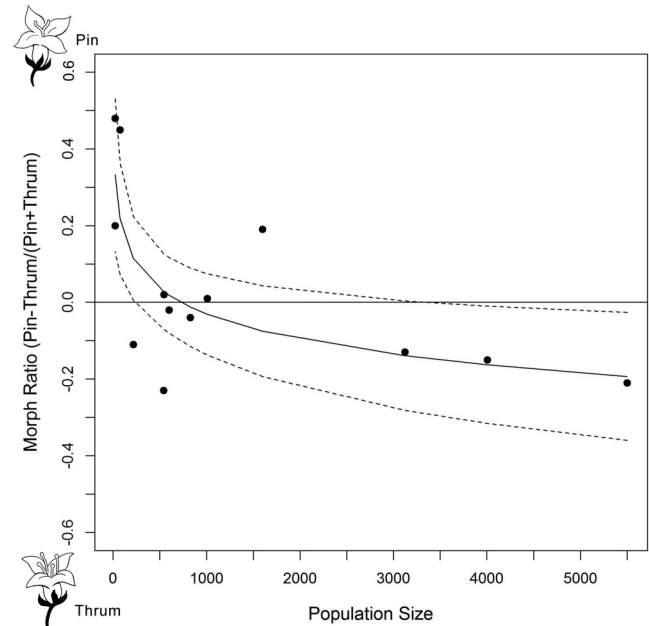
Akaike's information criterion (AIC) was used to identify the most parsimonious model (Akaike 1974) among the eight candidate models. We used a χ^2 test to determine if the most supported model differed significantly from the ecological base model. For the most supported model, we also calculated the correlation between observed and predicted values of seed production to assess model fit. Finally, to examine the nature of the influence of predictor variables on seed production, predicted values of the response variable were graphed for the most supported model. Observed values were used for the factors graphed, whereas all other factors were held constant at their means.

Results

Morph ratios ranged from -0.21 (more thrum than pin) to 0.48 (more pin than thrum) for the 13 populations (Table 1) with morph ratio being negatively related to population size ($\beta = -0.22$, $SE = 0.06$; $p = 0.003$, $R^2 = 0.56$). Small populations were pin-biased, whereas large populations were more even to slightly thrum-biased (Fig. 1). A population size of 726 individuals (95% CI = 236–3316) was predicted to result in an even morph ratio (Fig. 1).

The most supported model for seed production of *H. longifolia* was the incompatibility model including the interaction between morph type and proportion pin, plant size (no. of stems and maximum height) as a covariate, and location as a random effect (Table 3). This incompatibility model had significantly better fit than the ecological base model ($\chi^2 = 30.93$, $p < 0.001$) with change in AIC ($\Delta AIC = 25$) being large. In the incompatibility model, plant height, number of stems, and the interaction between morph type and proportion pin were positively related to seed production, with thrum morph and proportion pin negatively related to seed production (Table 4). Seed production in thrums increased with increasing proportion of surrounding pins, whereas seed production in pins decreased as the proportion of surrounding pins increased (Fig. 2). Pins had greater seed production than thrums across most pin proportions, with pins and thrums producing an equal number of

Fig. 1. Linear-log model of mean morph ratio in *Houstonia longifolia* by population size. Note: Morph ratio varies from -1.0 (only thrum morphs) to 1.0 (only pin morphs). Isoplethy is indicated by the horizontal line at 0.0 . The mean (linear-log regression) relationship between morph bias and population size is shown by the solid line with the upper and lower 95% CI indicated by the broken lines.



seeds when the proportion of pin plants was ~ 0.85 (Fig. 2). Thrums were able to produce seed in the absence of pins, but at lower levels than produced by pins in the absence of thrums (Fig. 2). The correlation between observed and predicted values of seed production for the most supported model (incompatibility model) was moderate ($r = 0.57$), suggesting reasonable predictive power for the model, but indicating the existence of other unmeasured or random factors affecting seed production.

Discussion

We investigated the reproductive ecology of *H. longifolia* to test hypotheses about the factors influencing seed production and to provide information to guide conservation strategies for this locally imperiled species. This study demonstrated that small populations of *H. longifolia* were likely to have flower morph ratios biased toward the pin morph and that seed production was influenced by the interaction between morph type and the surrounding proportion of pins. These results suggest pins are potentially more self-compatible than thrums, but that there is still a strong self-incompatibility system requiring intermorph crosses for high production of seeds.

The relationship between morph ratio and population size followed our predictions with reductions in population size leading to morph biases (Molano-Flores 2001; Endels et al. 2002; Kery et al. 2003), with small populations of *H. longifolia* being pin-biased and large populations being more even to thrum-biased. These results

Table 3. Comparison of eight candidate (negative binomial generalized linear mixed-effects) models used to predict seed production in *Houstonia longifolia*.

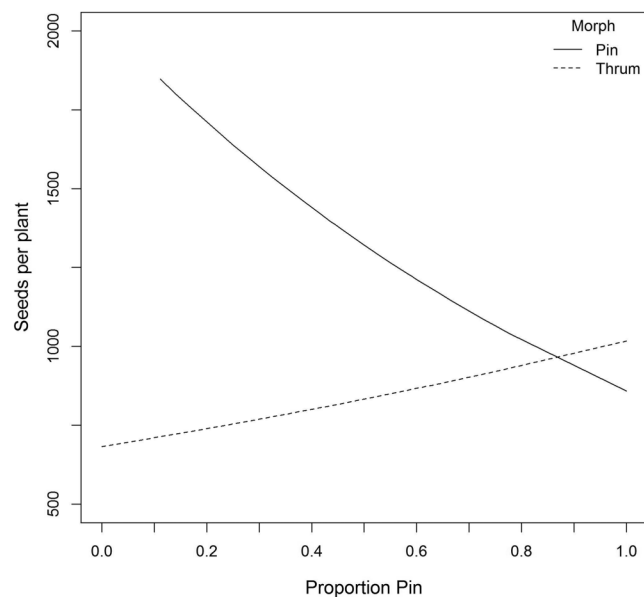
Hypothesis	Model structure	K	AIC	Δ AIC	w_i
Incompatibility	L+S+H+M×P	6	2814.6	0.0	0.463
Allee and incompatibility	L+S+H+N+M×P	7	2816.0	1.4	0.230
Density and incompatibility	L+S+H+D+M×P	7	2816.2	1.6	0.208
Allee, density, and incompatibility	L+S+H+N+D+M×P	8	2817.7	3.1	0.098
Density (local density)	L+S+H+D	5	2828.2	13.6	<0.001
Allee and density	L+S+H+N+D	6	2829.5	14.9	<0.001
Allee (populations size)	L+S+H+N	5	2838.1	23.5	<0.001
Ecological base	L+S+H	4	2839.6	25.0	<0.001

Note: L, location (random); H, maximum plant height; S, no. of stems; M, morph; N, population size; P, proportion pin; D, density. Δ AIC refers to the difference in AIC from the most supported incompatibility model; w_i refers to the Akaike weight of each model.

Table 4. Summary of estimates for different parameters in the most supported model (incompatibility model in Table 3) of seed production in *Houstonia longifolia*.

Fixed effect	β	SE
log(stems)	0.761	0.082
log(max. height)	1.054	0.234
Morph (thrum)	-1.092	0.207
Proportion of pin	-0.862	0.165
Morph (thrum): proportion of pin	1.261	0.351

Note: Plant size (stems and maximum height) included as a covariate with location as a random effect.

Fig. 2. The relationship between proportion of surrounding pins and seed production in *Houstonia longifolia* for plants of each morph type based on the most supported model (see Table 4). Other predictor variables in the model were held constant at their means.

also show that a loss of individuals from a small population is more likely to result in a morph bias than the loss of individuals from a large population. If a morph bias does occur, fewer intermorph crosses will take place and seed production will decline. Thus, intervention measures such as translocation of the less abundant morph

type or hand pollination between pins and thrums may be required (Ågren and Ericson 1996; Molano-Flores 2001). Therefore, maintaining an adequate population size is critical to ensure the persistence of this species. We estimate a minimum population size of 726 individuals (95% CI = 236–3316) would be needed to ensure even morph ratios; this can serve as a benchmark for managers to use in future conservation plans.

Both morph types were able to produce seed in the absence of the opposite morph suggesting this species does not have very strict self-incompatibility. However, the evidence that small populations were pin-biased and that pins had higher seed production than thrums in the absence of the opposite morph supports the suggestion that pins are more self-compatible than thrums. This greater self-compatibility in pins agrees with what has been found in other species (e.g., *Hedyotis nigricans*, *Pentas lanceolata*, *Jepsonia heterandra*, *Primula sieboldii*, and *Pulmonaria officinalis*) (Bahadur 1970a, 1970b; Ornduff 1971; Matsumura and Washitani 2000; Brys et al. 2008). However, future research is still needed to confirm the existence of this greater self-compatibility in the pin floral morph and to determine its strength and underlying mechanisms.

Our analysis revealed results similar to other studies in that larger populations had more even morph ratios (Kery et al. 2000; Matsumura and Washitani 2000; Jacquemyn et al. 2002; Kery et al. 2003). These even morph ratios are likely to result in greater rates of intermorph crosses leading, in turn, to higher seed production. This suggests the existence of a component Allee effect for this species through a mechanism of morph bias reproductive incompatibility. That is, the chance of finding a compatible mate (i.e., pin or thrum) increases as population size increases (Courchamp et al. 2008; Gascoigne et al. 2009) leading to greater seed production.

Surprisingly, our density hypothesis (local density) for seed production was less supported than the incompatibility hypothesis, even though numerous studies have shown that the proportion of intermorph crosses is positively related to plant density (Schaal 1978; Nicholls 1987; Shibayama and Kadono 2003). There are several possible explanations for this result. Firstly, this species

may experience a phenomenon known as “ideal free distribution” (Fretwell and Lucas 1969) in which pollination rates for each flower are equal regardless of the number of open flowers (i.e., local density of individuals with open flowers) (Dreisig 1995). Secondly, pollinators may have overlooked even dense patches of this small-statured plant to visit nearby more attractive plant species with larger floral displays (Yang et al. 2011; Seifan et al. 2014). Thirdly, the spatial pattern of individuals surrounding a focal plant may have influenced seed production more than the actual local density of individuals (Geslin et al. 2014; Seifan et al. 2014). Fourthly, the scale at which we measured local density (1 m²) may not have been the scale at which density affects seed production. Therefore, pollination levels, spatial pattern, and conspecific species surrounding *H. longifolia* should be investigated in future studies.

With distylous species experiencing heteromorphic incompatibility, seed production was predicted to vary by morph type and the proportion of surrounding morph types (Shibayama and Kadono 2003; Van Rossum et al. 2006; Brys et al. 2008). For the distylous species *Pulmonaria officinalis*, the short-styled morphs relative fecundity (i.e., ratio of mean seed set of short-styled morph over mean seed set of long-styled morph) was positively related to populations with greater long-style morph frequencies (Brys et al. 2008). Similar findings were obtained by Van Rossum et al. (2006) with seed set for *Primula veris* being positively related to proportion pins and varying among morph types. The most supported model (incompatibility model) confirmed these results for *H. longifolia* with the proportion of pins positively related to seed production in thrums and negatively related to seed production in pins. This result is the same even when the large population (~49 200 individuals) was included in this analysis (see Supplementary data for analyses including the largest populations)¹. This provides support for the existence of self-incompatibility in this species and illustrates how the occurrence of compatible mates (i.e., even morph ratios), which would increase the chances of intermorph crosses, results in higher seed production. This also indicates that if pin intramorph crosses occur more often as pin proportions increase, seed production for this species would most likely decline. Therefore, any translocation of this species for conservation purposes should consist of equal proportions of pins and thrums to increase the chance of intermorph crosses.

As expected, plant size was positively related to seed production and this can be attributed to the fact that taller, multistemmed plants are more likely to have multiple flowers. Data on number of flowers per plant were not collected due to the short flowering period causing a continual loss of flowers during the duration of these surveys. This increase in seed production for taller, mul-

tistemmed plants could be related to pollination levels. For instance, Schmitt (1983) found stalk height and number of flower heads affected pollinator selection. This pollination selection was also found for F₂ hybrids of dioecious *Silene* species, for which visit rates were greater for tall plants with multiple flowers than short plants with few flowers (Brothers and Atwell 2014).

Based on *H. longifolia*'s limited dispersal, current levels of habitat fragmentation within its range, and the expected rate of climate change, it has been ranked 10th most vulnerable to climate change out of 419 rare plant species assessed for Alberta (Barber et al. 2016). To prevent this species' decline, conservation strategies should formulate strategies that consider this species' incompatibility system and thus focus on the maintenance and establishment of large populations that contain even morph ratios.

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