Variation in restorer genes and primary sexual investment in gynodioecious *Plantago coronopus*: the trade-off between male and female function

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In many gynodioecious species the nuclear inheritance of male fertility is complex and involves multiple (restorer) genes. In addition to restoring plants from the female (male sterile) to the hermaphrodite (male fertile) state, these genes are also thought to play a role in the determination of the quantity of pollen produced by hermaphrodites. The more restorer alleles a hermaphroditic plant possesses, the higher the pollen production. To test this hypothesis I combined the results of crossing studies of the genetics of male sterility with phenotypic data on investment in stamens and ovules among the progeny of plants involved in these studies. The sex ratio (i.e. the frequency of hermaphrodites among the progeny), being a measure of the number of restorer alleles of the maternal plant, was positively related to the investment in pollen (male function), but negatively related to the investment in ovules (female function), in both field and greenhouse experiments. Consequently, a negative correlation between male and female function was observed (trade-off) and it is suggested that antagonistic pleiotropic effects of restorer genes might be the cause. Phenotypic gender, a measure combining investment in both pollen and ovules, was highly repeatable between field and greenhouse, indicating genetic determination of a more male- or female-biased allocation pattern among the studied plants.

Keywords: gender; hermaphrodite; pleiotropy; restorer genes; sex allocation; trade-off

1. INTRODUCTION

Gynodioecy was originally described as a system with two distinct gender classes: hermaphrodite (H) and male sterile (MS) (Darwin 1877). However, many researchers also noted the existence of partial-male-sterile (PMS) individuals, i.e. individuals with intermediate or mixtures of flower types (see for a summary Koelewijn & Van Damme 1996). These PMS plants constitute a continuous series, connecting complete male sterility to hermaphroditism. Moreover, within the group of hermaphrodites large variation in pollen production has been observed for several gynodioecious species, e.g. thyme, *Thymus vulgaris* (Atlan *et al.* 1992; Gigord *et al.* 1999); wild strawberry, *Fragaria virginiana* (Ashman 1999) and buck's-horn plantain, *Plantago coronopus* (Koelewijn & Hundscheid 2000). These observations support the suggestion of Lloyd (1980) that a continuous scale would more adequately represent the quantitative nature of plant sexuality.

Several studies show that the genetics of sex determination in gynodioecious species are complex. Male fertility is determined by multiple loci, both dominant and recessive (Van Damme 1983; Belhassen *et al.* 1991; Koelewijn & Van Damme 1995b; Charlesworth & Laporte 1998; Dudle *et al.* 2001). From their crossing studies, Koelewijn & Van Damme (1995b) concluded that MS individuals are not restored, PMS individuals are moderately restored and H individuals are highly restored at male fertility loci. They were able to link the sexual state of a plant to the number of restorer alleles. Moreover, Koelewijn & Van Damme

(1996) suggested that variation in pollen production within the class of H plants is also related to variation in restorer alleles. The more restoration at the male fertility loci, the higher the pollen production of an H. Thus, restorer genes could influence both the sex ratio of the progeny and their sex-allocation strategy.

In most gynodioecious species MS plants outperform H plants in one or several aspects of female reproduction (see reviews in Gouyon & Couvet 1987; Frank 1989). As MS plants (females) do not invest in male function, they might devote more resources to ovule or seed production. This resource-allocation mechanism has been called 'compensation' (Darwin 1877) and is at the heart of sex-allocation theory (Charnov 1982; Charlesworth & Morgan 1991; Campbell 2000). It also applies to sex allocation within the class of H plants if pollen production is related to the number of restored male-fertility loci.

I studied sex allocation in the class of H plants in *P. coronopus*, in the context of the genetics of male sterility. First, I evaluated the genetics of male sterility by determining the sex ratio (i.e. the H frequency) among the progeny of specific crosses. As part of this analysis, I assessed whether there is a relationship between the inferred number of restorer alleles of maternal plants, as derived from the crossing programme, and the sex ratio of their progeny. Second, I quantified the investment in anthers (male function) and ovules (female function) in the H progeny of these crosses and related this to the sex ratio, in both field and greenhouse studies. Third, I assessed whether there is a genetic trade-off between male and female functions in hermaphrodites. Fourth, I related phenotypic gender to the progeny sex ratio to test the hypothesis that maternal plants with many restorer genes produce progeny with a more male-biased sex-allocation

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Figure 1. Experimental crossing design. Two siblings, one MS (female) and one H (hermaphrodite), from the progeny of each of 10 individuals (grandmothers, further denoted as families) were crossed with each of three randomly chosen pollen donors.

pattern. Finally, I compared the phenotypic genders of field- and greenhouse-raised plants to determine the constancy of sex expression among families.

2. MATERIAL AND METHODS

(a) *Species*

Plantago coronopus is a herbaceous short-lived perennial rosette plant that occurs mainly in salt marshes. It is gynodioecious, with functionally hermaphroditic, PMS and MS individuals co-occurring within populations (Koelewijn & Van Damme 1996). Sex expression is under nuclear–cytoplasmic control (Koelewijn & Van Damme 1995a,b) with hermaphrodites being denoted as restored, i.e. their male function is restored by nuclear (restorer) genes. It is a wind pollinated and predominantly outcrossing species (outcrossing rate of 0.80; Koelewijn 1998). Each plant produces numerous flowers on top of long flowering stalks (spikes), which consist of a stem and an ear part. Perfect H flowers produce one ovary, containing five ovules and four bright yellow stamens.

(b) *Crossing design*

Seeds were collected from 80 flowering plants in a 400 m² square area within one population (Westplaat, 4°0.05' E 51°0.57' N; sex ratio (i.e. H frequency) of 0.82). Ten seedlings from each plant were grown in the greenhouse to select families that segregate both MS and H offspring. Owing to their segregation, these plants are known not to be fully restored at their male fertility loci. Sex ratios among the offspring varied from 0.20 to 1.00 with most families (65%) being fully restored. From 10 selected families one MS and one H sibling were crossed to each of three pollen donors, randomly taken from the remaining families. In addition, the hermaphrodite was self-fertilized. Thus, ten sets of seven crosses were obtained (figure 1). Each set has a common grandmother and will be denoted as a family and is the unit of comparison. A family therefore consists of a combination of maternal half-sibling crosses.

(c) *Genetics*

To obtain a sex-ratio estimate and to determine the genotypes of individuals, progeny from these crosses were grown in an experimental garden. Seeds were germinated in Petri dishes on moist filter paper. Seeds that did not germinate spontaneously

within one week were cut at the side of the radicle to stimulate germination. Seedlings were then transferred to jiffy pots filled with potting compost, grown in the greenhouse for four weeks and then transferred to the experimental garden. The progeny of each cross were divided into two batches, which were grown at different locations in the experimental garden: (i) to obtain a replicate of the sex-ratio estimate; and (ii) to correct for environmentally induced sex expression (Koelewijn & Van Damme 1996).

Flowering started within six to eight weeks of planting. Mean survival from seed to flowering was 91.2%. Sex-type scores were based on 20–50 flowering spikes per plant. Sex-ratio estimates were based on ca. 68 plants (range of 28–128 plants). A χ^2 -test was used for testing the difference between observed and expected segregation ratios.

(d) *Sex allocation*

To estimate primary investment in both female (ovules) and male (stamens) functions in H plants, the progeny of the crosses were used in both a field and a greenhouse experiment. Owing to a shortage of seeds in some families, only four out of the six crosses were used.

In February 2000, seeds from each family mother-cross combination ($n = 40$) were placed in Petri dishes with moist filter paper in a climate chamber (16 L : 8 D photoperiod; 20 °C during the day, 15 °C at night; photosynthetic photon flux density (PPFD) of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). After germination the seedlings were planted in trays filled with washed dune sand and grown outdoors for one month. Seedlings were transferred to the field at the beginning of March. The experiment was carried out at the Westplaat, the site from which the maternal parents originated. A homogeneous part of the salt marsh measuring 8 m × 6 m was selected and divided into 12 square plots (2 m × 2 m). Each cross was represented in each plot by two seedlings. Seedlings were randomized within a plot and placed 5 cm apart in a hexagonal design. During the summer of 2000 three flowers from the first flowering spike of each of four hermaphrodites from each cross were collected from plants that flowered within a time span of two weeks. By doing this I minimized the confounding influences of time- and age-dependent sex allocation (H. P. Koelewijn, unpublished data).

The greenhouse experiment was set up in a similar way, except that all plants were grown according to a randomized design and six H plants per cross were used. Seeds were germinated on moist filter paper in Petri dishes, and after 14 days seedlings were transferred to pots containing 700 mg of washed river sand and placed in the greenhouse. Plants received 25 ml of a half-strength Hoagland nutrient solution every week and were watered regularly. Flowers were collected from the first flowering spike. All plants flowered within a time span of 14 days.

Collected flowers were located just above the flowers with already dehiscent anthers. Flowers were dissected and stamens and ovules were carefully removed. The carbon content was determined using a Unicarb carbon analyser (Salonen 1981). From each flower only one of the four stamens was used as variation among anthers within a flower is small (H. P. Koelewijn, unpublished data).

To measure the sex of individual plants, I used Lloyd's (1980) concept of standardized phenotypic gender. Gender is herein defined as the proportion of an individual's total fitness derived through male function

Table 1. Example of a set of crosses used in determining the relationship between the average number of restorer alleles and the H fraction among the offspring (see figure 2).

(Two siblings, one H and one MS, were each crossed with each of the same three fathers (pollen donors). Moreover, the hermaphrodite was self-fertilized (see figure 1). Indicated in the table are the proposed genotypes of the parents, the number of MS and H individuals among the offspring, the fraction of H individuals, the χ^2 -test for deviations from the fitted ratio (with d.f. = 1), the significance level (p : n.s., not significant) and the average number of restorer alleles among the offspring. The proposed model involves one dominant and two recessive restorer genes. Note that hermaphrodite h17-1 has an MS genotype, indicating the presence of different cytoplasmic types in the population.)

cross B 071–B 077 (family p9)										
cross	female parent	male parent	genotype (male)	MS	H	% H	fitted ratio	χ^2	p	no. of restorers
<i>hermaphrodite p9-2: proposed genotype R1+ +r2 +r3</i>										
B 077	p9-2	p9-2	R1+ +r2 +r3	17	104	0.86	9 : 55	0.00	n.s.	3
B 076	p9-2	p7-2	R1+ +r2 +r3	11	70	0.84	9 : 55	0.16	n.s.	3
B 074	p9-2	h17-1	++ +r2 ++	25	31	0.55	3 : 5	0.93	n.s.	2
B 075	p9-2	h6-2	R1+ r2r2 r3r3	4	45	0.92	1 : 15	0.09	n.s.	4
proportion of H among offspring of plant p9-2						0.79				
<i>male sterile p9-1: proposed genotype ++ +r2 +r3</i>										
B 073	p9-1	p7-2	R1+ +r2 +r3	20	61	0.75	9 : 23	0.38	n.s.	2.5
B 071	p9-1	h17-1	++ +r2 ++	64	24	0.27	3 : 1	0.14	n.s.	1.5
B 072	p9-1	h6-2	R1+ r2r2 r3r3	8	43	0.84	1 : 7	0.33	n.s.	3.5
proportion of H among offspring of plant p9-1						0.62				
proportion of H among offspring of family p9						0.71	total χ^2 (d.f. = 6)	2.03	n.s.	

Table 2. Results from a log-linear analysis of maternal sex, family and father-within-family effects on the proportion of hermaphrodites in the progeny of crosses.

(Model indicates the error term used for calculating the significance of a treatment. n.s., not significant.)

source	d.f.	deviance	mean deviance	F	p	model
1. maternal sex	1	150.8	150.8	24.72	***	5
2. family	9	963.1	107.1	7.52	***	3
3. father within family	20	284.8	14.2	8.35	***	6
4. maternal sex by family	9	26.1	2.9	0.48	n.s.	5
5. maternal sex by father	20	122.4	6.1	3.59	***	6
6. residual	60	101.0	1.7			

*** $p < 0.001$.

$$A = \frac{m_i/M}{(m_i/M) + (f_i/F)},$$

where f_i and m_i are the female and male fitnesses of individual i , and F and M are the total female and male fitnesses in the population. Phenotypic gender can vary from zero (all female) to one (all male), and is 0.5 for an entire population. Because I did not measure fitness directly, I used investment in either stamens or ovules to characterize phenotypic gender.

(e) Data analyses

The proportion of H progeny in each cross was analysed by fitting a log-linear model with a binomial error distribution using GLIM (Baker 1987). The analysis started with a null model with all main effects and interactions. Subsequently, a χ^2 -test was used to determine whether dropping a term from the model significantly reduced the explained variance. The difference in unexplained variance between the categorical models (deviance) is approximately χ^2 -distributed, with the number of degrees of freedom equal to the difference between the models with and without the term to be tested (McCullagh & Nelder 1983).

Because there were often significant interactions, the significances of the main effects were estimated using F -statistics with the deviance of the main effect (divided by the degrees of freedom) as the numerator and the deviance of the interaction (divided by the degrees of freedom) as the denominator (cf. Crawley 1993).

Continuous variables were analysed using analysis of variance by the GLM procedure of SAS (SAS Institute 1989). The basic approach was first to test for a difference among the 40 crosses, as a first indication of the presence of genetic variation, and subsequently to divide the sum of squares into its components. Family and father within family were considered random effects, sex was fixed. Data from the field were log-transformed to achieve normality and homogeneity of variances.

3. RESULTS

(a) Genetics

I was able to assign genotype satisfactorily for individuals from seven out of the 10 original sets of crossings.

Table 3. Results from analyses of variance (ANOVA) of the carbon contents of anthers and ovules and of phenotypic gender in (a) the field and (b) the greenhouse.

(Model indicates the error term used for calculating the significance of a treatment. n.s., not significant.)

			anthers (μg C)		ovules (μg C)		gender	
source	model	d.f.	mean squares	p	mean squares	p	mean squares	p
(a) field								
1. between crosses	2	39	0.338	**	0.382	n.s.	0.298	**
4. maternal sex	7	1	0.817	n.s.	0.107	n.s.	0.161	n.s.
5. family	6	9	0.511	**	0.277	n.s.	0.690	**
6. father within family	2	10	0.110	n.s.	0.279	n.s.	0.126	n.s.
7. maternal sex by family	8	9	0.279	n.s.	0.486	n.s.	0.176	n.s.
8. maternal sex by father	2	10	0.393	**	0.473	n.s.	0.185	n.s.
2. individuals within crosses (experimental error)	3	118	0.179	***	0.341	***	0.152	***
3. flowers within individuals (residual error)		284	0.011		0.018		0.012	
(b) greenhouse								
1. between crosses	2	39	0.233	***	0.366	***	0.039	***
4. maternal sex	7	1	0.112	n.s.	0.005	n.s.	0.041	n.s.
5. family	6	9	0.295	n.s.	0.757	*	0.096	*
6. father within family	2	10	0.271	*	0.162	n.s.	0.031	*
7. maternal sex by family	8	9	0.182	n.s.	0.270	n.s.	0.026	n.s.
8. maternal sex by father	2	10	0.123	n.s.	0.178	n.s.	0.024	n.s.
2. individuals within crosses (experimental error)	3	205	0.115	***	0.149	***	0.014	***
3. flowers within individuals (residual error)		451	0.016		0.026		0.002	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Figure 2. Relationship between the (inferred) number of restorer alleles and the sex ratio (H fraction) among the progeny of MS $\times$ H (filled circles) and H $\times$ H (open circles) crosses.

The fitted models indicate both dominant and recessive restorer-gene action and the presence of multiple genotypes within both the H and the MS phenotypic classes. An example is given in table 1.

The sex ratio varied greatly among the 70 crosses (range of 0.02–1.00), between families (range of 0.41–0.91) and between maternal sex types (H, 0.82; MS, 0.66; table 2). These results indicate substantial variation in the number of restorer alleles among individuals within a natural population.

With up to five restorer alleles, there was a clear relationship between the average number of restorer alleles among the offspring (as estimated from the fitted models) and the sex ratio (figure 2). Variation in the sex ratio can

therefore be used as an indicator of the number of restorer alleles present in maternal plants.

(b) *Investment in male or female function*

Investment in anthers and ovules differed significantly among crosses in the greenhouse. In the field only a significant difference only in investment to anthers was observed (table 3). These results indicate the presence of genetic variation in both reproductive traits. Anthers and ovules were much larger in the greenhouse than in the field (anthers: field 22.8 ± 6.2 , greenhouse 32.4 ± 7.1 ; ovules: field 11.2 ± 3.8 , greenhouse 16.1 ± 4.0 ; mean ($\mu\text{g C}$) ± 1 s.d.; $n = 243$ (greenhouse) or $n = 158$ (field)). No effect of maternal sex on the performance of their H offspring was observed (table 3). Significant differences between families for anthers (field) and ovules (greenhouse) were observed, indicating the presence in the population of maternal genotypes with more male- or female-biased allocation patterns (table 3).

(c) *Sex ratio and reproductive investment*

In the field and in the greenhouse the sex ratio of families was positively correlated with male investment, but negatively correlated with female investment (figure 3). Although the differences between families were small, of the order of 20–30% (anthers: field, 17.2–25.5 $\mu\text{g C}$; greenhouse, 27.7–34.7 $\mu\text{g C}$; ovules: field, 9.8–13.3 $\mu\text{g C}$; greenhouse, 13.8–19.0 $\mu\text{g C}$), they changed consistently with the sex ratio, causing moderate to strong correlations at the family level. Owing to the opposing relations of male and female investment to sex ratio, a negative correlation between investments in male and female functions at the family level was observed (figure 4).

Figure 3. Relationships between sex ratio among the progeny of 10 families and the anther (filled circles) and ovule (open circles) weights of the H offspring of these families (mean $\pm$ 1 s.e.). Indicated are the best fit from linear regression and the Pearson correlation coefficient. All correlation coefficients were significant at least at the $p < 0.05$ level. (a) Field, (b) greenhouse.

(d) Gender variation

Gender or maleness differed significantly between families (table 3). The average maleness of the offspring of each of the 10 families was positively correlated with the sex ratio (figure 5). Thus, hermaphrodites from families with a low sex ratio had a more female-biased sex-allocation pattern, and those with a high sex ratio were more male biased. Also, a strong positive correlation between maleness estimates from field and greenhouse was observed (figure 6). The results indicate that maleness or gender (i.e. relative investment) of the progeny is strongly determined by the maternal genotype, even when the plants are grown in environments that cause differences in absolute investment, and indicate that the population consists of families with more male- or female-biased sex-allocation patterns.

4. DISCUSSION

(a) Genetics of male fertility

In line with previous genetic studies in the genus *Plantago* (Van Damme 1983; Koelewijn & Van Damme 1995b; de Haan *et al.* 1997) and other gynodioecious species (e.g. oregano, *Origanum vulgare* (Kheyr-Pour 1980); *T. vulgaris* (Belhassen *et al.* 1991); bladder companion, *Silene vulgaris* (Charlesworth & Laporte 1998); great blue lobelia, *Lobelia siphilitica* (Dudley *et al.* 2001)) the results presented here indicate complex nuclear inheritance of male fertility. Hermaphrodites are restored at multiple loci, and both

Figure 4. Relationships between anther and ovule weights (mean $\pm$ 1 s.e.) for plants grown in the field (open circles) and greenhouse (filled circles). Indicated are the best fit from linear regression and the Pearson correlation coefficient. Both correlation coefficients were significant at the $p < 0.05$ level.

dominant and recessive restorer genes are involved. Elucidating the detailed genetics of sex determination in gynodioecious species is therefore laborious and involves many crosses. Several authors have therefore suggested the use of the H fraction (sex ratio) of the progeny of a maternal plant as an indicator of the number of restorer alleles present in that plant (Gigord *et al.* 1999; Taylor *et al.* 2001; Bailey 2002). Here, I provide support for this suggestion. For up to five restorer alleles there is a clear relationship between the average number of restorer alleles among the offspring of a cross and the sex ratio of that cross (figure 2). When the average number of restorer alleles among the offspring is five or more, the sex ratio will be one and all progeny will be hermaphrodites. With such a high number of restorer alleles individuals are likely to be homozygous at at least one of the restorer loci. These results suggest that the best strategy to get an idea about variation in restorer alleles among plants in natural populations is to cross a large number of maternal plants with a random sample of pollen donors from the same population. The differences in progeny sex ratios would then reflect differences in the numbers of restorer alleles possessed by the maternal plants. The drawback, compared with crossing studies, is that no detailed genetic interpretation is possible. The advantage is that a large number of plants can be screened in this way, thus providing more information at the population level (cf. Taylor *et al.* 2001).

(b) Restorer genes and reproductive functions

Investment in male function by hermaphrodites was positively correlated with progeny sex ratio. The greater the H frequency within the progeny, the greater the pollen production of the flowers of those hermaphrodites. A similar result was obtained in *T. vulgaris* (Gigord *et al.* 1999). Thus, the better restored families contained hermaphrodites with greater male reproductive function. This result is in line with the observations of Govinda Raj & Virmani (1988) that pollen production increased with the number of restorer alleles present in rice (*Oryza sativa*). By

Figure 5. Relationship between the sex ratios of the progeny of 10 families and the gender (maleness, mean $\pm$ 1 s.e.) of the H offspring of these families. Correlation coefficients were 0.95 and 0.92 for the field (open circles) and greenhouse (filled circles), respectively (both significant at $p < 0.01$).

contrast, investment in female function was negatively correlated with progeny sex ratio. This suggests that the genes that restore male fertility influence both female and male fertility in hermaphrodites. In general the female reproductive function of hermaphrodites may be limited by the amount of reproductive resources used for male function, resulting in a negative correlation between the allocations to the two sexual functions (Charnov 1982; Charlesworth & Morgan 1991; Campbell 2000).

Despite the (theoretical) trade-off between male and female reproductive functions, positive correlations are often detected where negative correlations were expected (see Campbell 2000). Differences between individuals in the amount of resources available (Van Noordwijk & de Jong 1986), variation in resource acquisition (Houle 1991), the use of different currencies (Ashman 1994) and the experimental genetic design used (Campbell 1997; Mazer *et al.* 1999) have all been put forward as explanations for this incongruity. In this study I specifically chose to estimate primary sexual investment, i.e. the investment in anthers and ovules in the first flowering spike. At this stage both sexual functions are likely to draw on the same resource pool. Moreover, I used the same currency for both functions (investment in carbon). Finally, I measured sexual investment by using the mean value of six maternal half-sibling crosses, which is the most reliable estimator of the additive genetic covariance (Becker 1985; Mauricio & Mojonner 1997). In doing so, both in the field and in the greenhouse, a negative correlation between the two sexual functions was detected, thus providing evidence for the basic assumption of sex-allocation theory (Ashman 1999; Campbell 2000). However, it should be kept in mind that the individuals used in this study were selected because they were known to have variation in their restorer genes. This variation was also expected to be reflected in their H offspring. Because the absolute differences between families in investments in male and female functions were small, this approach might make it easier to detect the trade-off between the two sexual functions. Families that do not segregate male steriles are expected to be fully restored and differences

Figure 6. Maleness of the H offspring of 10 families raised in the field and the greenhouse. The dotted line is the line of equality ($r = 0.92$, $p < 0.01$).

between these families might be even smaller, making it much more difficult to detect a trade-off.

The opposing relationships between both male and female reproductive functions and family sex ratios suggest that restorer genes are the cause of the negative correlation between the two sexual functions. Thus, the nuclear genes that act on the restoration of male function also act on the efficiency of reproductive function, by changing the sex-allocation pattern of H plants. Differences in the reproductive outputs of MS and H plants are often interpreted as a pleiotropic effect of the cytoplasmic MS gene: by not producing pollen, male steriles save resources for a higher seed output (Kohn 1989; Delph 1990; Ashman 1994; Poot 1997). The present study shows that this effect is not restricted to a difference between sex morphs, but might also apply within the H class, suggesting that there is a gradual change in female and male allocation from male sterile to hermaphrodite for *P. coronopus*.

(c) *Quantitative determination of sexual phenotypes*

The large variations in reproductive functions in hermaphrodites and their correlation with sex ratio suggest quantitative determination of gender in *P. coronopus*. This study was concerned with variation in allocation at the flower level. In the case of pure hermaphrodites this will also reflect allocation at the plant level. Gender or maleness of individual plants varied from 0.21 to 0.83, indicating a continuum in male allocation among the H plants. In PMS plants, allocations at the flower and plant levels will be different, with the magnitude of the difference depending on the proportion of sterile flowers produced. Moreover, even within the class of MS plants differences in anther size have been observed (Koelewijn & Van Damme 1995a), also giving rise to subtle allocation differences. Thus, if gender is expressed as the ratio of male investment to female investment, a continuum exists at the plant level in *P. coronopus* when all phenotypic sex morphs are combined (cf. Koelewijn & Van Damme 1996).

Families showed small differences in phenotypic gender between field and greenhouse despite a twofold difference in absolute investment in ovules and stamens between the

environments. This indicates that stamens and ovules were equally affected by differences between the two environments (correlation coefficient field–greenhouse ($n = 10$): stamen 0.87, $p < 0.01$; ovule 0.64, $p < 0.05$) and that genotypes try to maintain the same ratio of male to female investment irrespective of environmental conditions. The existence of genotypes with a more male- or female-biased allocation pattern within a population will give rise to sexual asymmetry and continuous frequency-dependent selection (Ross 1990), thus generating possibilities for the maintenance of genetic variation in reproductive characters.

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As this paper exceeds the maximum length normally permitted, the author has agreed to contribute to production costs.