Mapping fitness landscapes to interpret sex allocation in hermaphrodites

Kai-Hsiu Chen*, John R. Pannell

Department of Ecology and Evolution, University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland

* Kai-Hsiu Chen

Email: kai-hsiu.chen@unil.ch

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Abstract
Sex allocation theory successfully predicts sex-ratio variation among organisms with separate sexes, but it has been much less successful in explaining variation in sex allocation in hermaphrodites because the assumption of a direct tradeoff between male and female functions is often violated. Here, we show that sex-allocation theory can be applied to hermaphrodites simply by mapping components of seasonal reproductive success onto a fitness landscape defined by potentially independent measures of allocation to male and female functions on orthogonal axes. Taking this approach allowed us to interpret the complex variation in the reproductive strategy of a long-lived perennial herb (*Pulsatilla alpina*) that produces both male and bisexual flowers and that shifts between male and female allocation among seasons. We find that components of reproductive success for *P. alpina* map onto a rugged landscape with peaks that reflect an interactive effect of male and female allocations on self-fertilization and total reproductive success and that correspond to the observed sex-allocation strategies adopted by the species in nature. This simple approach should be widely applicable to problems in the study of hermaphroditic reproduction in other plants and animals.

Significance Statement
Sex allocation theory has helped to explain sex-ratio variation in numerous dioecious species, but it has been difficult to apply to hermaphrodites, in which male-female tradeoffs are often obscure. Here, we show that by mapping fitness estimates for plants with complex allocation patterns on a two-dimensional landscape defined by both male and female allocations, we sidestep the tradeoff assumption. Our analysis reveals fitness peaks that correspond precisely to the strategic allocation decisions adopted by the species in nature. Our simple but novel approach provides a rescue-line for a powerful body of theory that has been criticized for being too difficult to apply to the messy world of hermaphrodites, both in plants and animals.

Introduction

The theory of sex allocation has provided a simple and powerful framework for understanding the reproductive strategies of species with two different gamete sizes (1). The theory explains when a species should be dioecious versus hermaphroditic (2), what sex ratio dioecious species should have under different scenarios of inbreeding, kin competition or conflict (3, 4), whether sex should be determined by genes or by the environment (5), what proportion of reproductive resources hermaphrodites should allocate to their two sexual functions (2, 6), whether hermaphrodites should allocate to both sexes simultaneously or sequentially (7), and whether (and how) their allocation strategy should depend on their size (8, 9). But whereas sex-allocation theory has been extraordinarily successful in predicting the sex ratio and timing of allocation to sons versus daughters in species with two separate sexes, it has been notoriously difficult to test in populations that include hermaphrodites (10–15) (but see sex change in hermaphroditic animals (16)).

Applying sex-allocation theory to hermaphrodites is difficult not only because we have so few estimates of hermaphrodite fitness for both male and female functions (13, 15), but especially because individuals are assumed to divide their reproductive allocation between their male and female functions in a zero-sum game, yet such sex allocation tradeoffs remain elusive. The obscurity of simple sex-allocation tradeoffs is likely the result of differences in resource availability among individuals (17), but also due to the fact that male and female functions in many hermaphrodites likely draw on resources that do not fully overlap (18). In some plants, for example, pollen production is likely limited by nitrogen availability whereas fruit production is more limited by carbon (19, 20), so the straightforward tradeoffs assumed by theory should not be expected. Regardless of the underlying reason, the obscurity of sex-allocation tradeoffs in hermaphrodites (21) has limited the use of an otherwise highly successful theory to understand the complexity of their reproductive strategies – such as variation in allocation within and among reproductive seasons and especially, in plants, among inflorescences and flowers of the same genetic individual.

Here, we decipher the complexities of sex allocation in perennial hermaphrodites by taking the simple step of allowing the male and female allocations of hermaphrodites to vary independently of one another in their effects on fitness (as opposed to being constrained by a tradeoff). We showcase our approach by modelling fitness components of a long-lived hermaphrodite, the self-compatible insect-pollinated herb *Pulsatilla alpina*, in terms of male and female allocations, both on their own as single variables and jointly. Although individuals may produce up to about twenty male and/or bisexual flowers, those in our study population were small and mainly single-flowered, allowing us to use genetic markers to assign paternity to individual flowers and so determine how within-flower sex allocation affects fitness gains. Sex allocation is already highly variable among *P. alpina* flowers, but we further enhanced this variation by removing stamens from a subset of flowers in the population (Figure 1). This allowed us to infer the potential fitness also of female phenotypes that do not occur in the wild, presumably because they are selected against.

We first modelled estimates of male and female fitness in terms of components of sex allocation to characterize 'fitness gain curves' that relate fitness through each sexual function to allocation to that same function. The shape of such gain curves has been widely invoked to explain the evolutionary stability of uni- versus bisexuality (1, 6) and is thought to be largely saturating in hermaphroditic populations (10). In contrast, our analysis clearly points to an accelerating fitness gain curve for female function in *P. alpina*. We then mapped fitness components onto a two-dimensional fitness landscape defined by male and female allocations on orthogonal axes, revealing fitness peaks that correspond closely to the complex sex-allocation strategy adopted by *P. alpina* in the wild. Our study not only succeeds in providing one of the first characterizations of an accelerating fitness gain curve in a hermaphrodite organism, but also demonstrates a simple

and empirically accessible way that might help to comprehend particularly complex sex-allocation strategies in hermaphroditic species generally.

Results

Variation in floral sex allocation and components of reproductive success

We measured the male and female components of sex allocation for a total of 175 flowers, with 104 individuals producing a single flower and 31 individuals producing more than one flower (Figure S1). Prior to the stamen removal manipulations, 46 and 129 flowers were male and bisexual, respectively. Note that experimental stamen removal did not alter the flowering duration of the flowers (22). We then estimated female and male components of seasonal reproductive success for all individuals that had only one flower (Figure S2). Each seed family produced 90.5 ± 55.4 mature seeds (mean ± SD). We could assign paternity for 854 seeds to a single most likely father under a relaxed confidence interval (80%), corresponding to a 96% successful assignment rate for what amounted to around 9% of all mature seeds produced in the population. Using selfing rates estimated for the seed families (22), and assuming a value of inbreeding depression of d = 0.95 estimated on the basis of comparisons of inbreeding coefficients between adults and seed progeny (23), we inferred the mean female, male, and total reproductive success of single-flowered individuals to be 41.4 ± 48.6, 33.6 ± 41.5, and 75 ± 61.3, respectively (Supplementary Information Figure S2). These values compare with corresponding estimates of 65.8 ± 58.4, 57.9 ± 51.9, and 123.7 ± 88.3, respectively, under the assumption of a hypothetical scenario of d = 0. The comparison of fitness estimates that consider inbreeding depression with those that do not is valuable, because most fitness estimates published to date have ignored the potential effects of inbreeding depression (24–26), yet it is clear from our comparisons that inbreeding depression may strongly affect inferences of selection acting on relevant traits.

Female and male floral fitness gain curves

We estimated the shape of the male and female gain curves for individual flowers on the basis of reproductive success estimates for the 87 parents in our sample that produced a single flower (Figure 2; 17 flowers were aborted or missing). Regression of our estimate of female fitness on pistil production within flowers clearly points to an accelerating female fitness gain curve (exponent b = 1.91 ± 0.3; mean ± se; significantly > 1.0; Figure 2A). The accelerating female gain curve can be attributed to the effects of sex allocation on the selfing rate and its deleterious effects on progeny fitness: increased relative female allocation means that, on average, each pistil receives less self-pollen. The significance of selfing and inbreeding depression in shaping the gain curve can again be seen in the contrast with the hypothetical scenario that assumes no inbreeding depression, where we infer a slightly saturating gain curve for the female function (b = 0.85 ± 0.16; Figure 2C), though the curve does not differ statistically from linearity (b is not significantly different from 1.0). In contrast to the inferred accelerating female fitness gain curve, our data point to strongly saturating gain curves for male function, whether incorporating our estimate of inbreeding depression (b = 0.47 ± 0.38; Figure 2B) or not (b = 0.52 ± 0.27 (Figure 2D), though again neither of these two gain curve estimates differed significantly from linearity.

Linear, quadratic, and correlational selection gradients on female and male allocation

We further performed a selection gradient analysis (27), which fully confirmed the above results: female reproductive success was a clearly accelerating function of allocation when incorporating inbreeding depression in our fitness estimates, but linear when inbreeding depression was ignored (Figure 3A). Similarly, male reproductive success was a largely saturating function of allocation, whether inbreeding depression was considered or not (Figure 3E and Supplementary Information Table S1). Our selection gradient analysis for female reproductive success also points to mostly negative directional selection on male allocation when fitness estimates incorporate inbreeding depression (Figure 3D and Supplementary Information Table S1), likely as a result of ovule discounting (28). For male reproductive success, our results point to stabilizing selection on female allocation, but only when inbreeding depression is ignored (Figure 3B and Supplementary Information Table S1). Total reproductive success depended only on female

allocation, though on both female and male allocation when ignoring the effects of inbreeding depression (Figure 3C and F; and Table S1). We could detect no correlational selection on female and male allocations (correlational selection gradients did not differ significantly from zero; Table S1).

Two-dimensional fitness landscapes for female, male, and total reproductive success
Maps of female, male, and total reproductive success on a two-dimensional sex-allocation landscape are presented in Figures 4A, B, and C, respectively. The two fitness peaks in the figure for total reproductive success correspond closely to phenotypes of unisexual male and bisexual flowers, respectively (Figure 4C). Standardized linear, quadratic, and correlational selection gradients, estimated using the *GSG* package for *R* (29), are presented in Table S2. Note that we detected evidence for negative correlational selection gradients for total reproductive success when incorporating inbreeding depression in our fitness estimates (Table S2).

Discussion

Estimation of both male and female gain curves in a natural population of flowers
Our study provides a rare empirical estimate of the shape of fitness gain curves for a natural plant population. It has hitherto been difficult to estimate the shape of fitness gain curves for several reasons, not least because most species do not display a sufficiently wide range of phenotypic variation in natural populations over which to estimate fitness. By removing some or all of the stamens of a sample of flowers of *P. alpina*, we extended the already wide range of sex allocation represented in the population to include not only male and bisexual flowers, which occur naturally, but also flowers that were fully female. Because the range of sex allocation in the study population covered the full plane of allocations, we could estimate male and female gain curves relatively independently of one another.

Our analyses indicate that the male fitness gain curve for *P. alpina* was saturating. Although there have been very few empirical estimates of the shape of the male gain curve for insect-pollinated plants (13, 14), those that do exist have also found evidence for saturating male gain curves (30–32, but see 33). Saturating fitness gain curves are expected if pollen accumulation saturates on pollinators' bodies (13), or if pollen from a given individual is delivered to a small number of receptive stigmas, causing local mate competition (6, 34) or local sperm competition (15). *P. alpina* is pollinated almost exclusively by flies that cause substantial within-flower self-pollination (35) and that disperse pollen among individuals over short distances (36), conditions that should give rise to local mate competition and thus to saturating male fitness gain curves.

In contrast with the saturating male gain curve, the female gain curve for *P. alpina* was strongly accelerating. The female gain curve is often suspected to be a saturating function in plants, not accelerating, because of likely competition among progeny dispersed into a limited seed shadow ('local resource competition') (13). While we did not measure possible effects of local resource competition on the gain curve, we believe this possibility is negligible in *P. alpina* because the single-seeded fruits ('achenes') are furnished with parachute-like structures that aid wide dispersal by wind from elongated floral stalks (37, 38). Significantly, although pollen is dispersed over very short distances, there is almost no genetic structure in populations of *P. alpina* (36), confirming that seeds are likely well-dispersed. The accelerating fitness gain curve estimated for *P. alpina* is probably due to the fact that flowers with relatively more pistils and fewer stamens have a lower selfing rate and produce progeny that are thus protected from expressing high inbreeding depression (35). This explanation is confirmed by analysis that ignores the effects of inbreeding depression, which predicted a slightly (albeit not significantly) saturating female gain curve rather than the accelerating curve predicted by analysis that incorporates inbreeding depression into fitness estimates.

To our knowledge, this is the first study to have directly inferred an accelerating fitness gain curve for female reproductive success in a plant species, and, moreover, for reproductive success at

the flower level. The possibility of a dependence of female fitness on male allocation was addressed by Charlesworth and Charlesworth (2) in a model linking sex allocation theory to the realities of mixed mating in hermaphrodites, as well as by de Jong et. al. (39) in a model conceiving the selfing rate as a positive function of male allocation under 'mass-action' assumptions (40). The idea that increased allocation to male function (both the production of more pollen but also allocation to attractive structures) can increase the selfing rate and, through the expression by progeny of inbreeding depression, reduce the female component of reproductive success, has been influential in shaping our understanding of the evolution of floral strategies. However, this explanation has largely addressed the effects of selfing caused by pollen transfer among flowers of the same individual ('geitonogamy') (41–43). By studying a population of plants producing mainly single flowers, we were able to discern the fitness implications of sex allocation at the flower level. Although within-flower selfing is thought to contribute a great deal to the total selfing rate of many self-compatible hermaphrodite species (35, 42, 44), it has hitherto not widely been possible to determine the relationship between within-flower allocation and the mating system (and fitness) (35).

A landscape approach for interpreting complex sex allocation strategies

Because components of allocation to male and female reproduction in *P. alpina* vary relatively independently of one another, we could ask whether male and female fitness components might depend on allocation to the other sexual function in ways that go beyond linear sex-allocation tradeoffs. We indeed found that the selfing rate and a flower's contribution to female reproductive success was a function not only of the number of pistils, but also of the number of stamens for a given pistil number (35). Classic sex allocation theory is unable to explain such patterns in a straightforward way, yet they are likely to be common in perennial plants and animals whose resource status varies with size or for other, more cryptic, reasons (15, 45).

To appreciate the value of mapping fitness components on a two-dimensional landscape of empirically measurable components of sex allocation in species in which variation in resource status obscures potentially underlying tradeoffs, consider the diagonal lines traced on the fitness maps inferred *P. alpina* in this study, depicted in Figure 4A-C. These lines represent a potential tradeoff between the male and female allocations of individuals with the same resource availability, with a class of relatively small, or low-resourced, individuals occupying the bottom-left diagonal, and increasingly larger individuals occupying the middle and upper-right diagonals. Figures 5A and B depict the hypothetical female and male fitness gain curves corresponding to each of these three resource-level scenarios, while Figure 5C plots total fitness gains as a function of both male and female functions. These plots show how the shape of fitness gain curves may vary with plant resource status. In the case of our data for *P. alpina*, the tradeoff lines traced over the fitness landscape (Figure 4) reveal variation in the shape of the fitness curves (Figure 5) that suggest that small individuals should allocate most of their reproductive resources to their male function, while larger individuals with more resources should allocate substantially to both male and female functions. With growth, individuals should thus shift from an all-male to a hermaphroditic allocation strategy, i.e., they should display a type of 'sexual diphasy' (46–48), as indeed observed in wild populations of *P. alpina* and many other perennial plants (49–51) and animals (52).

The existence of two fitness peaks on the sex allocation landscape for *P. alpina* (Figure 4) also points to 'andromonoecy' as a successful strategy, with some flowers adopting a fully male and others a hermaphroditic strategy. Again, this pattern corresponds to the strategy actually adopted by *P. alpina.* In general, it is plausible that individuals with substantial resources available for reproduction might use as much as they can for the production of bisexual flowers and whatever remains for the production of a male flower. It has also been found that, in andromonoecious species of *Solanum*, there is a strong correlation between the size of the fruit and the fraction of male flowers, supporting the notion that male flowers in andromonoecious species may often serve to balance sex allocation (53).

Finally, the topography of the fitness landscape provides a clear indication of a saturating (rather than the otherwise accelerating) female fitness curve for flowers of *P. alpina* from which stamens had been experimentally removed in our study, i.e., for a phenotype that does not occur in nature (because *P. alpina* flowers always bear stamens; Figure 4A). Here, it is satisfying to note that *P. alpina* stamens function not only to sire ovules but also to attract pollinators to flowers that do not produce nectar, so that the lower female reproductive success of flowers without stamens may have been a result of pollinator limitation – though this conjecture needs testing. The function of stamens as both the source of pollen for siring offspring as well as a reward for pollinators might further help to explain why the flowers of *P. alpina* always produce stamens and thus why the species is andromonoecious and not monoecious.

Conclusion

Sex allocation theory has been successful in helping to explain variation in sex ratios in dioecious species, particularly animals, but its quantitative application to hermaphrodites has so far largely failed – for both operational and conceptual reasons. Our study has overcome key operational difficulties by successfully estimating both the male and female contributions to fitness by floral modules across their full potential range of allocations. It also showcases a simple but potentially useful approach for interpreting complex patterns of sex allocation in terms of the shape of a fitness landscape defined by independent measures of male and female allocation on orthogonal axes. This approach may provide an empirically accessible rescue-line for a body of powerful theory that has been increasingly criticized for being too difficult to apply to the messy world of hermaphroditic reproduction (13, 15, 54–56). Specifically, our study shows how this approach allows complex hermaphroditic strategies to be explained in terms of sex allocation, after all.

Materials and Methods

Study species and study sites

We studied a population of *Pulsatilla alpina* (L.) Delarbre (Ranunculaceae) at Solalex in the pre-Alps of Vaud canton, Switzerland ('Population S1+'; latitude: 46°17′42″N, longitude: 7°09′09″E; elevation: 1758 a.s.l.) in the spring and summer of 2022. The species grows in sub-alpine to alpine habitats in central Europe, with longevity likely exceeding 30 years (57). Each spring, several vegetative and/or reproductive shoots emerge from a rhizome soon after the snowmelt, with single flowers on separate flowering shoots. Small plants often produce a single male flower, while larger plants produce up to about 20 usually bisexual flowers, i.e., the species displays quantitative gender diphasy (58). Bisexual flowers have a similar number of stamens to that of male flowers and up to 400 pistils (Figure 1) (22). Flowers are predominantly visited by flies, including houseflies and syrphid flies (38). Ripe fruits (technically achenes) with elongated pappus hairs are dispersed by wind in early autumn (37). *P. alpina* is self-compatible and has a selfing rate of about 0.4, with the rate of selfing being a positive function of within-flower male allocation and selfed progeny expressing high inbreeding depression 0.95 (35). We studied 135 mainly single-flowered individuals on an open slope of sub-alpine grassland that we fenced to exclude cattle and other browsers. We removed all floral buds from the few unsampled individuals outside the plot at the beginning of the flowering season to prevent them from siring progeny in the plot.

Flowering phenology

We recorded the location and flowering state of all individuals in the population from late May to late June, 2022, noting the number and sexual phenotype of their flowers. For each flower and sampling date, we recorded each flower's sexual stage in terms of seven and five ordinal categories for bisexual and male flowers, respectively (detailed description of the categories in ref. (22)). These categories allowed us to manipulate and estimate sex allocation for flowers comparable at the same developmental stage (see Results).

Manipulation of floral sex allocation
Although flowers of *P. alpina* vary widely in their sex allocation, natural variation does not include flowers with few or no stamens. To estimate the potential fitness contributions of female or nearly fully female flowers, and thus to evaluate the full two-dimensional fitness landscape, we removed stamens of a sample of flowers at times throughout the flowering season, as has been done in other studies (54, 59, 60). At each observation time point, we randomly selected about a quarter of the bisexual flowers in their early female stage and used tweezers to remove 100% or 50% of their stamens (treatments SR_{100} or SR_{50}, respectively). Similarly, we removed 50% of the stamens of about a quarter of male flowers in their early male stage (treatment SR_{50}) (22). Stamen removal did not alter the flowering duration of the flowers (22). Because it occurred before anthers opened, there was no accidental intra-flower selfing.

Estimates of floral sex allocation
We quantified floral sex allocation to the two sex functions as the number of stamens and pistils produced by each flower. For male allocation, we photographed all hermaphroditic and male flowers at the late female stage and the mid_male stage (detailed description of the stages in ref. (22)), respectively, and later counted the number of stamens on the basis of the photographs calibrated against measures for a sample of 15 fresh flowers (58). weeks after flowering ended, flowers with developing fruits were enclosed in individual paper bags to prevent seed dispersal, and seeds were later collected for counting. We quantified female allocation as the total number of achenes in each flower (38).

Paternity assignment and estimates of floral selfing rates
We assigned paternity to each of ten seeds sampled randomly from each flower in our sample, using variation at ten microsatellite loci and the software Cervus v 3.0.7 set with a confidence level of 80% and an error rate of 0.018 (see details in ref. (22)). Individuals whose flowering timespan did not overlap with the focal bisexual flower were excluded from the list of candidate fathers for each of the seed families genotyped. In total, 892 of 1054 sampled seeds could be genotyped for at least five loci and used for paternity analysis (PCR was unsuccessful for the remaining seeds and were not included).

Estimates of female reproductive success
We sorted 22,612 achenes from 104 seed families (19 and 6 seed families from 129 bisexual flowers were aborted or missing, respectively) into unfertilized, predated, and mature seed categories, following ref. (38). We then calculated components of reproductive success assuming both the estimated value of inbreeding depression (d = 0.95), as well as assuming no inbreeding depression (d = 0), with female reproductive success computed as the inferred number of mature outcrossed seeds plus ($1 - d$) times the inferred number of mature seeds produced by selfing, based on the selfing rate estimated for each flower.

Estimates of male reproductive success
We calculated male reproductive success for each flower as the number of outcrossed seeds sired on other individuals in the population plus the number of seeds sired by selfing multiplied by ($1 - d$), again for both d = 0.95 and 0.0, as described before. Because we genotyped about ten seeds for all flowers, irrespective of the total number of seeds produced by the flower, we estimated the male reproductive success of a given potential sire by multiplying the fraction of the seeds in the flower it sired by the total number of seeds in that flower.

Statistical analysis
We related prospective reproductive success to sex allocation using nonlinear least square models (*nls* function in R *stats* (61)) to evaluate the shape of fitness gain curves for female and male functions at the flower level, assuming both d = 0 or d = 0.95. The gain curve f was modelled as $f = ax^b$, where, x is the number of pistils or stamens for the female and male

functions, respectively, a is a constant, and $b < 1$ and $b > 1$ correspond to a saturating or accelerating dependence of fitness on sex allocation (60, 62).

We also used conventional selection gradient analysis with multivariate regression to evaluate the dependency of reproductive success on both the female and male components of allocation (i.e., as two phenotypic traits), including second-order polynomial and interaction terms under the two scenarios of inbreeding depression at the flower level (27). We used linear regression models (*lm* function in R *stats* (61)) to evaluate the dependency of components of reproductive success on female and male allocations. We standardized female and male reproductive success as the mean for individuals and fitted this standardized value as a response variable. We standardized pistil number and stamen number to a mean of zero and a standard deviation of one, setting linear, quadratic, and interaction terms for the two traits to evaluate linear and non-linear (i.e., quadratic and correlational) selection gradients on female and male allocation (27, 63). For all quadratic gradients, we multiplied the regression coefficients by two to obtain the correct estimate of stabilizing or disruptive selection (64).

Finally, we used nonparametric regression with smoothing functions to characterize the fitness landscapes for reproductive success in terms of female and male allocations under the two inbreeding depression scenarios (29, 65). Here, we used generalized additive models (*gam* function in R package *mgcv* (66)) for the dependency of components of reproductive success on female and male allocations on pistil and stamen number, assuming a Poisson error distribution for the response variable. We applied the *gam.gradients* function in the *R* package *GSG* to extract standardized linear, quadratic, and correlational selection gradients from the fitted models and calculated the standard errors and *P* values on the basis of 1,000 bootstraps (29). To plot the fitness surface, we used a smoothing term with thin plate splines (66).

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Figure Legends

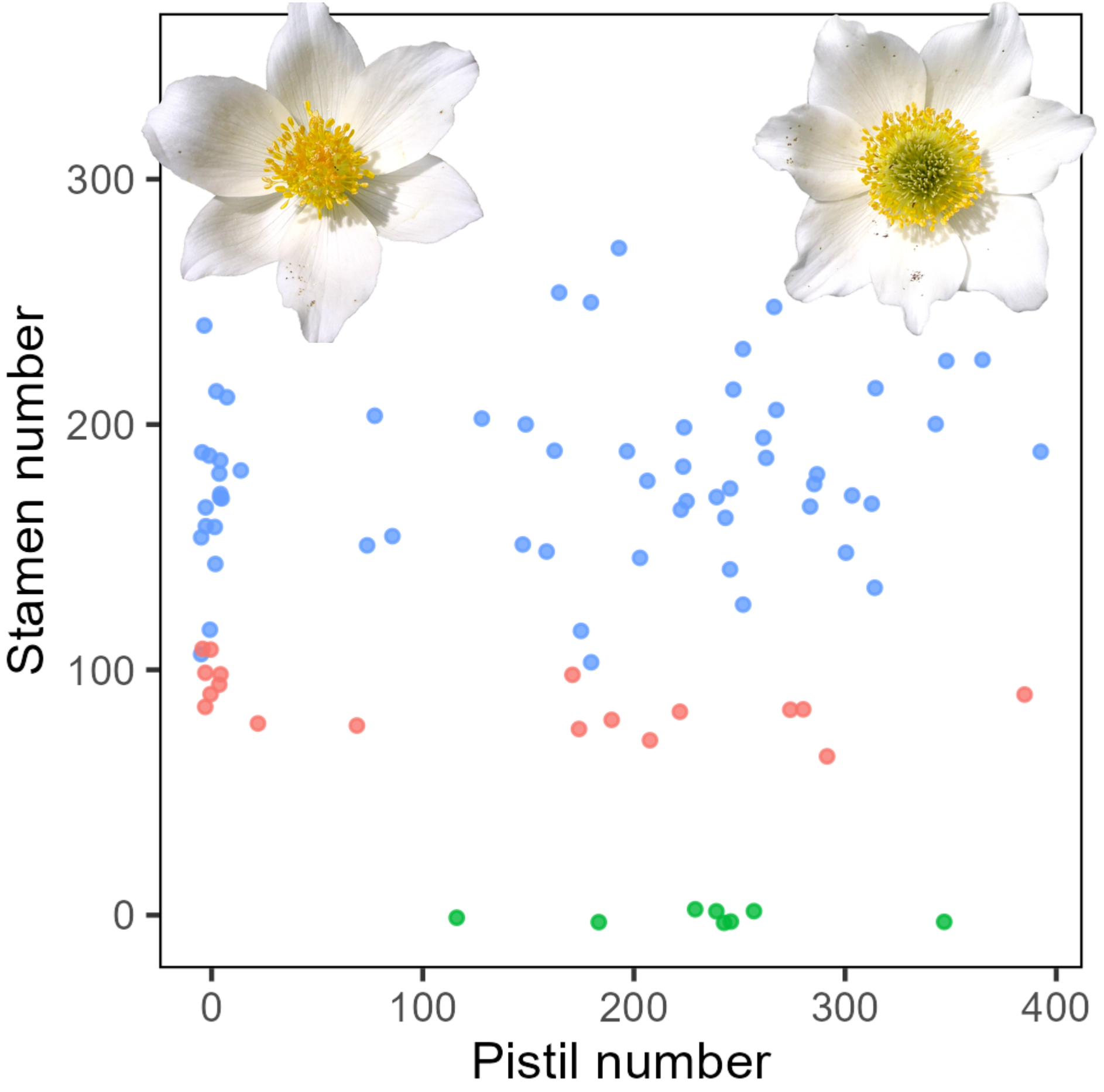
Figure 1. Morphological space of stamen and pistil number of the single-flowered individuals after stamen removal treatments. Green, orange, and blue points represent all-stamen-removed, half-stamen-removed, and intact flowers, respectively (*N* = 8, 18, and 61 respectively).

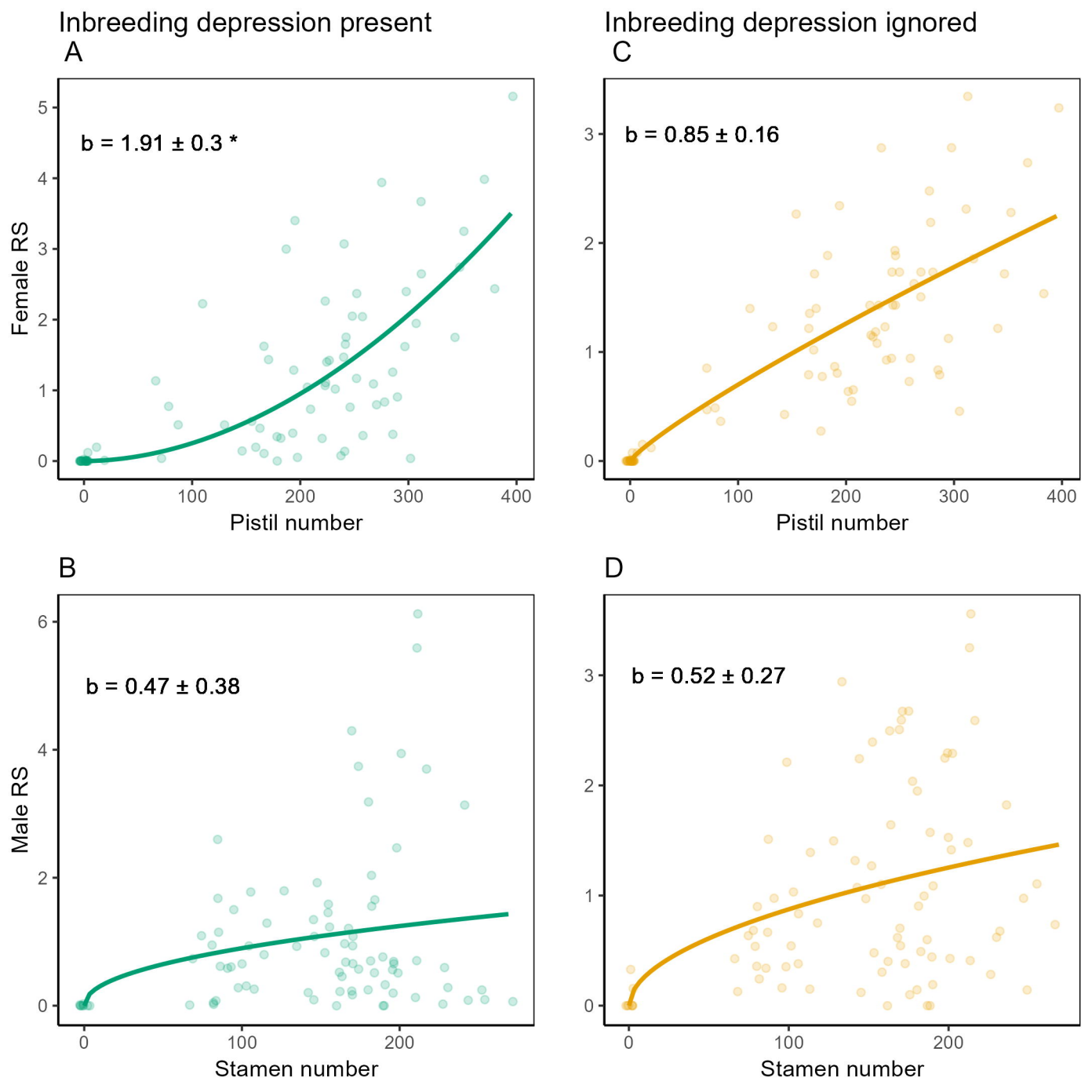
Figure 2. Fitness gain curves of female (upper panels) and male functions (bottom panels) at the floral level under the condition when inbreeding depression is taken into account (left-hand panels) or not (right-hand panels). Each point represents one individual with one flower (*N* = 87). The points were jittered to avoid overlapping. The shape of the gain curves was estimated by fitting exponential curves (see materials and methods for details) and the exponent *b* is shown in the figure with the standard error. An asterisk denotes that the curve was significantly non-linear.

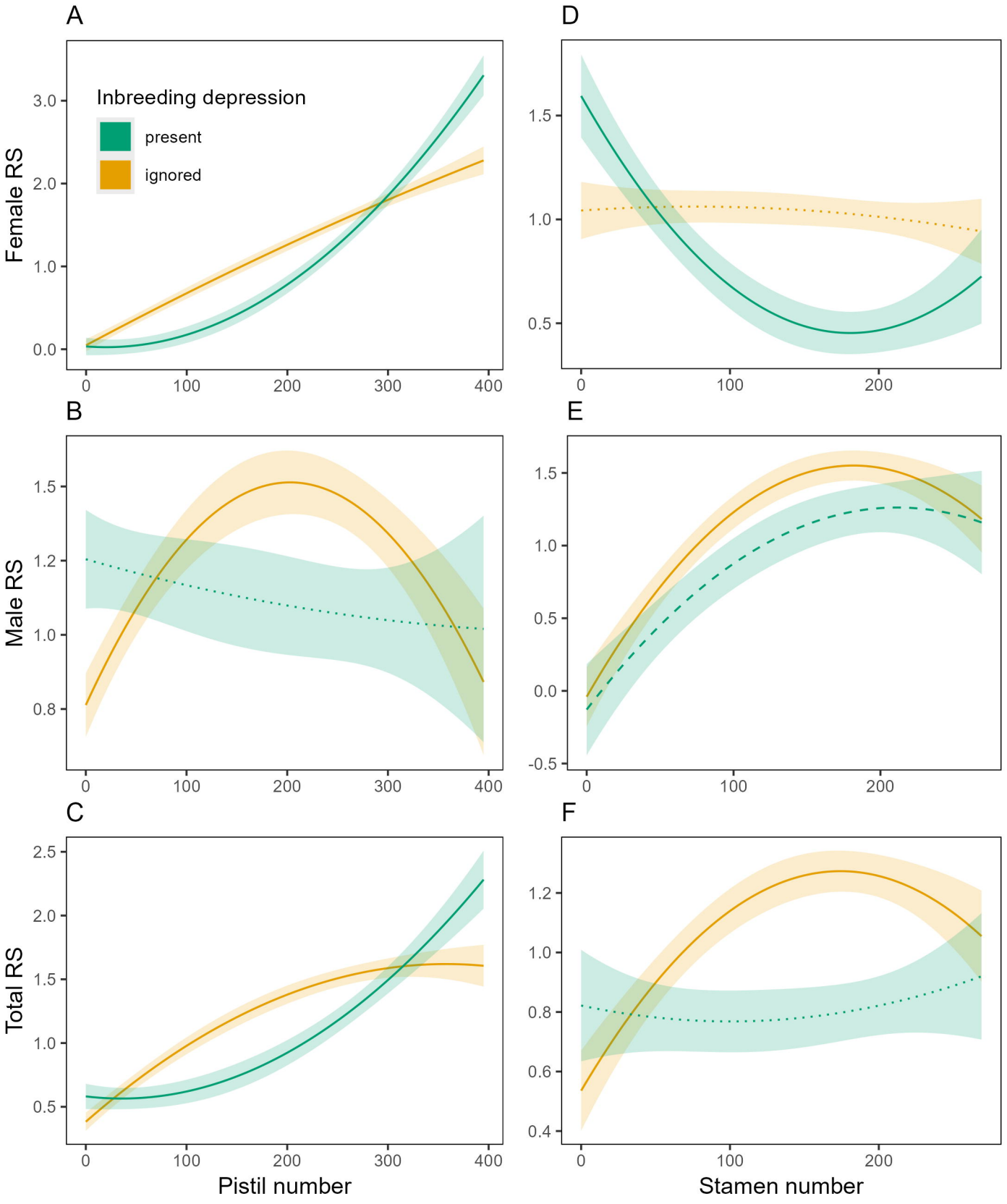
Figure 3. The dependence of female (upper panels), male (middle panels), and total (bottom panels) reproductive success (RS) on female and male allocation under the condition when inbreeding depression is taken into account (green lines) or ignored (yellow lines), estimated by selection gradient analyses (*N* = 87, see materials and methods for details). The shaded ribbon indicates the standard error of the regression curves. Regression lines of non-significant and marginally non-significant dependency of reproductive success on the sex function are shown in dotted and dashed lines, respectively.

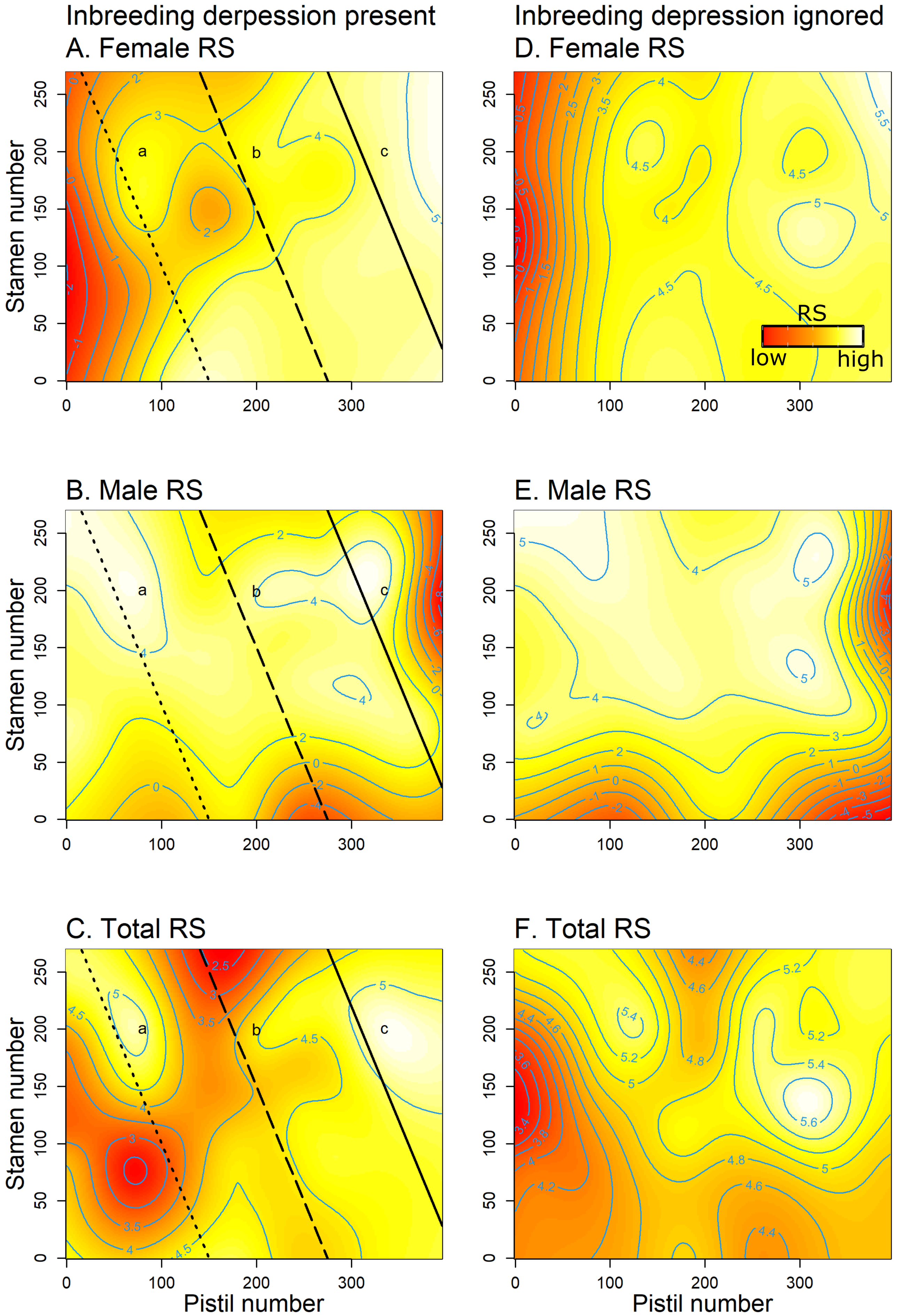
Figure 4. Representations of the fitness landscape for female, male, and total reproductive success (RS) as a function of pistil and stamen number in a flower under the condition when inbreeding depression is taken into account (left-hand panels) or ignored (right-hand panels), predicted by generalized additive models (*gam*) using 87 individuals with a single flower. The color gradient from red to white represents low to high predicted reproductive success. Hypothetical linear tradeoff lines between male and female functions were depicted by dotted (line a), dashed (line b), and solid lines (line c) for individuals of low, medium, and high resource status, respectively (Panels **A**-**C**). Note that the slope of the trade-off lines is conceptual because we do not know the actual trade-off ratio of one female and male unit. Individuals with a given amount of resource are only able to explore the left and bottom part of the trade-off line on the fitness landscape. Variance explained by fitted models for female, male, and total reproductive success were 79.9%, 58.2%, and 53.4%, respectively, when considering the influence of inbreeding depression, and 85.7%, 63.3%, and 67.5% when inbreeding depression was ignored.

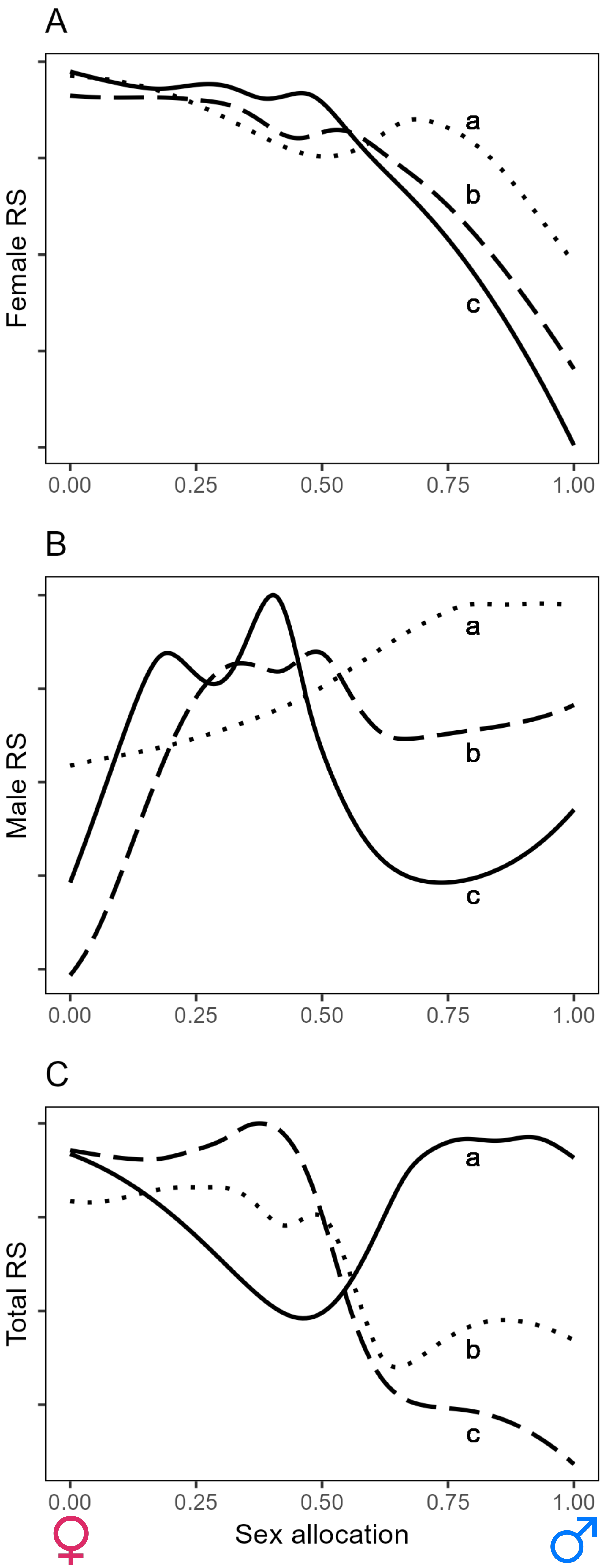
Figure 5. Conceptual figures demonstrating how female (**A**), male (**B**), and total (**C**) fitness gain curves depend on the resource status of an individual based on the study of *P. alpina*. Relationship of the reproductive success and sex allocation to the male function along the trade-off lines a, b, and c were extracted from Figure 4 **A** - **C**. Sex allocation in terms of maleness was calculated by dividing the stamen number by the sum of stamen and pistil numbers.


Stamen number
300
200
100
0
0
100
200
300
400
Pistil number


Inbreeding depression present
A
b = 1.91 ± 0.3 *
Female RS
Pistil number
Inbreeding depression ignored
C
b = 0.85 ± 0.16
Pistil number
B
b = 0.47 ± 0.38
Male RS
Stamen number
D
b = 0.52 ± 0.27
Stamen number


A
D
B
E
C
F
Inbreeding depression
present
ignored
Female RS
Male RS
Total RS
Pistil number
Stamen number
0.0
1.0
2.0
3.0
0.5
1.5
0.8
1.2
-0.5
0.4
0.6
2.5
0
100
200
300
400


Inbreeding derpession present
A. Female RS
Inbreeding depression ignored
D. Female RS
B. Male RS
E. Male RS
C. Total RS
F. Total RS
Stamen number
Pistil number
a
b
c
RS
low
high


A
Female RS
a
b
c
0.00
0.25
0.50
0.75
1.00
B
Male RS
a
b
c
0.00
0.25
0.50
0.75
1.00
C
Total RS
a
b
c
0.00
0.25
0.50
0.75
1.00
Sex allocation