Ken N. Paige · Barry Williams · Tracey Hickox

Overcompensation through the paternal component of fitness in *Ipomopsis arizonica*

Abstract Estimates of the effects of herbivory on plant fitness based on female fitness alone may be misleading if plants experience either reduced or increased male fitness. Because there are many plants that produce more flowers following herbivory where seed set is unaffected or reduced, total fitness may be enhanced through the paternal component alone. Here we show that herbivory results in an increase in reproductive success due solely to an increase in paternal fitness in the monocarpic biennial *Ipomopsis arizonica*. These results suggest that overcompensation may be more common than presently thought, requiring a reexamination of the fitness consequences of herbivory for many plant species.

Keywords Overcompensation · Ungulate herbivory · *Ipomopsis aggregata* · Paternal fitness

Introduction

Whether the direct effects of herbivory can enhance plant fitness has been contentious among ecologists and evolutionary biologists (Belsky 1986, 1987; Bergelson and Crawley 1992; McNaughton 1986; Paige 1994). Resolution has, until recently, been hampered by a lack of supportive evidence. Several studies, beginning in the late 1980s, provided experimental evidence of enhanced fitness (overcompensation) following herbivory in a natural system (Lennartsson et al. 1997, 1998; Paige and Whitham 1987a). Thus, debates have shifted from the existence of overcompensation to concerns about its relative importance, given the limited number of examples (Whitham et al. 1991). It is possible that many cases of overcompensation have been overlooked. To date, the majority of studies on plant-herbivore interactions have focused on the maternal, as opposed to the paternal, side of fitness (Gronemeyer et al. 1997; Strauss et al. 1996). Because there are many plants that produce more flowers following apical damage where fruit and seed set is reduced or unaffected (Hendrix 1979; Inouye 1982; Lowenberg 1994; Paige 1999; Reichman and Smith 1991), the paternal component of fitness could be enhanced, resulting in an increase in total fitness. We provide the first example of such a case. Using a combination of experimental and genetic approaches, we show that herbivory on *Ipomopsis arizonica* results in an overall increase in reproductive success through the paternal as opposed to the maternal component of fitness.

Materials and methods

Study sites and organisms

Field studies were conducted during the summer of 1997 in a population of approximately 500 *Ipomopsis arizonica* near Sunset Crater National Monument northeast of Flagstaff, Arizona (elevation, 2,300 m). At this site *I. arizonica* grows among stands of ponderosa pine, *Pinus ponderosa*, in soils largely consisting of volcanic cinder (68% of the mass per sample is made up of particles larger than 2 mm in diameter) (Maschinski and Whitham 1989). Plants were relatively evenly distributed across the site in open areas free of intra- and inter-specific competitors.

I. arizonica is a monocarpic, hermaphroditic herb that flowers from mid-late July through mid-late September. Following germination, *I. arizonica* develops into a leafy rosette. Although *I. arizonica* has an indeterminant vegetative phase as a rosette, it typically elongates and flowers in its second year (Maschinski and Whitham 1989; Paige and Maschinski, personal observations). Because it reproduces only once it is ideal for acquiring measures of lifetime fitness. *I. arizonica* is self-incompatible and pollinated primarily by two species of hummingbird: the rufous hummingbird, *Selasphorus rufus*, and the broad-tailed hummingbird, *Selasphorus platycercus* (Maschinski and Whitham 1989).

I. arizonica is browsed by mule deer, *Odocoileus hemionus*, during the period of stem elongation in late May. Ungulates remove approximately 95% of the above-ground biomass, browsing plants down to a height of 1–2 cm (Maschinski and Whitham 1989). Removal of the single inflorescence results in the production of multiple inflorescences from dormant lateral buds at the base of the plants stem (Maschinski and Whitham 1989). Ungulate herbivory does not result in a delay in reproduction to some subse-

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quent year once the plant has started to bolt. In addition, no ungulate herbivory occurs on rosettes prior to stem elongation.

Effects of ungulate herbivory on paternal fitness

To assess the effects of ungulate herbivory on paternal fitness, the following two experiments were conducted: a hand-pollination experiment and a natural pollination experiment followed by a paternity analysis. In addition, the following floral traits were measured to assess potential herbivore-induced changes in floral morphology that could affect pollen transport: corolla length, stigma and style length at receptivity, placement of the anthers (insertion and exsertion), and width of the corolla opening. Measurements were taken on 2 flowers from each of 25 randomly selected browsed and 25 randomly selected unbrowsed plants.

Hand-pollination experiment

The hand-pollination experiment was designed to assess the effects of pollen quality, as measured by the number of seeds sired per fruit, seed size, and germination success, between browsed and unbrowsed "males". This hand pollination experiment, by design, ignores potential differences in pollen production and floral morphology between browsed and unbrowsed fathers that could affect pollen transport. For this experiment, 36 focal "female" plants were selected in a systematic fashion such that plants were evenly spread across the entire population. Two "male" plants, one browsed and one unbrowsed, were selected approximately 10 m from each focal female. A male was only crossed with one female. All plants were selected prior to bud break and covered with nylon tricot-covered wire cages to exclude pollinators and herbivores. Following bud break, receptive stigmas (tri-lobes spread open) from four or more flowers were hand-saturated with pollen until each focal female set two fruits from each paired male. Fruits were bagged with nylon-tricot mesh and collected at the time of dehiscence. Complete data sets where fruits were sired by both browsed and unbrowsed "males" were retrieved from 17 of the 36 "female" plants for analysis (i.e., 17 browsed and 17 unbrowsed males nested within 17 females). Numbers of seeds per fruit were counted and seeds were individually weighed. Seeds were then germinated on no. 1 Whatman filter paper in moist petri plates to assess germination success. A split-plot analysis was conducted on number of seeds produced per fruit and seed weight.

Natural pollination experiment

The natural pollination experiment and genetic analysis was designed to assess the effects of herbivory on paternal fitness (number of seeds sired) under natural conditions. This experiment was conducted in an isolated meadow approximately 0.4 km from the study population. A small existing population of *I. arizonica* was clipped back to avoid contaminating the experimental population with foreign genes. Ten plants, five browsed and five unbrowsed, were transplanted from the study population into 1 gallon pots and placed in the meadow prior to flowering. Translocated plants were selected at least 10 m apart to minimize genetic similarities (Campbell 1991) and were matched for size (using stem diameter, Gronemeyer et al. 1997; Paige 1994). Upon flowering, pollinators were allowed to feed over the entire season. Following fruit-set, a subset of fruits were bagged with nylon-tricot mesh and upon dehiscence fruits and maternal tissues were collected for paternity analysis.

Following collection, parental leaf material was placed on dry ice, shipped to the University of Illinois and stored at –70°C until extraction. DNA was extracted from each of the 10 maternal plants and 33 offspring (an average of 3.3 per plant, 3.6 for browsed and 3.0 for unbrowsed dams), grown from collected seed, using CTAB (Weising et al. 1995). Following extraction, each DNA was quantified and diluted to a concentration of 25 ng/μl for PCR. Each PCR reaction contained 0.5 U of *Taq* polymerase, 0.1 mM of each dNTP, 0.2 μM of an Operon RAPD primer (from kits A & C), 20 mM Tris-HCl, 50 mM KCl, 2 mM MgCl$_2$, 25 ng of DNA and sterile water to a final volume of 25 μl and subjected to a "hot start" (Chou et al. 1992). The samples were then subjected to the following thermocycling conditions: (1) 3 min at 94°C, followed by 44 cycles of (2) 30 s at 94°C, (3) 30 s at 36°C, (4) 1.5 min at 72°C, and (5) a final extension for 10 min at 75°C (Williams et al. 1990). The end product was run on a 1% agarose gel stained with ethidium bromide and visualized under UV light. To assign parentage, gels were then photographed and scored for parental bands unique to either browsed or unbrowsed individuals.

Pollen production and viability

Potential confounding effects of differences in pollen production and/or pollen viability were assessed by collecting 56 flowers, 2 from each of 14 randomly selected browsed and 2 from each of 14 unbrowsed plants at the initiation of anther dehiscence for pollen counts and viability assessments. Flowers were collected from a separate set of plants from those used in the experiments described above. Pollen counts were conducted using a Beckman Z1 Coulter Counter (Harder et al. 1985). Pollen viability was assessed using a nitro blue tetrazolium stain (Hauser and Morrison 1964).

Effects of ungulate herbivory on maternal fitness

An assessment of maternal fitness was also made to confirm previous results (Maschinski and Whitham 1989) that browsed plants equally compensate in terms of maternal fitness (fruit and seed production). Fifty-five plants (25 browsed and 30 unbrowsed controls) were randomly selected and matched for size using stem diameter. These plants were compared in terms of numbers of flowers produced mid-season, during the peak of pollinator activity. Hummingbird pollinators peak in mid-season (late July) and rapidly decline throughout the month of August as they emigrate southward (Paige and Whitham 1985), dropping in population size by an average of 59% from mid to late season each year (Paige and Whitham 1987b). Flower and fruit production from a subset of these individuals (11 browsed and 10 unbrowsed) were collected at the end of the flowering season (late September). A second data set collected in 1987 on flower and fruit production from 18 individuals (9 browsed and 9 unbrowsed), matched for size early in the season, were used as an additional comparison to assess the generalizability of previous results (Maschinski and Whitham 1989) that browsed plants equally compensate in terms of maternal fitness.

Results and discussion

Effects of ungulate herbivory on paternal fitness

Hand-pollination experiment

Results of the hand-pollination experiment showed that there were no significant differences between seeds sired by browsed and unbrowsed males in the number of seeds produced per fruit ($F=0.185$, $df=1,15$, $P=0.674$) or seed mass ($F=0.754$, $df=1,15$, $P=0.399$; Table 1). This was true whether browsed or unbrowsed males were crossed with either browsed or unbrowsed females ($F=0.002$, $df=1,15$, $P=0.962$ for number of seeds produced per fruit; $F=0.437$, $df=1,15$, $P=0.519$ for seed weight). In addition, there were no significant interactions between browsed and unbrowsed females and browsed and un-

Table 1 Two experiments conducted to assess the effects of ungulate herbivory on paternal fitness traits in *Ipomopsis arizonica*. In experiment 1, browsed and unbrowsed males were crossed with a single browsed or unbrowsed female to assess components of male fitness. In experiment 2 a natural pollination experiment followed by a paternity analysis was used to assess the effects of herbivory on paternal fitness under field conditions. Means ±1 standard error of the mean are shown

	Browsed males	Unbrowsed males	P
Experiment 1 – Hand-pollination			
Seeds/fruit			
Browsed females	8.35±1.04	6.85±1.27	NS
Unbrowsed Females	6.43±1.43	8.93±1.95	NS
Seed mass (mg)			
Browsed females	1.21±0.15	0.98±0.16	NS
Unbrowsed females	1.20±0.13	1.24±0.17	NS
% Germination			
Browsed females	56.6%	56.1%	NS
Unbrowsed females	70.1%	67.6%	NS
No. pollen grains/flower	9,852±1131	8,767±789	NS
Pollen viability/flower	84.4±2.84	81.4±3.17	NS
Experiment 2 – Paternity analysis			
Predicted offspring sired	17.63	8.37	
Observed offspring sired	18.0	8.00	NS

browsed males for either number of seeds per fruit or seed weight ($P>0.11$). Furthermore, there were no significant differences between seeds sired by browsed or unbrowsed males in percent germination; this was true whether browsed and unbrowsed males were crossed with browsed females (56.6% vs 56.1% germination for browsed vs unbrowsed males, $\chi^2=0.008$, $df=1$, $P=0.95$) or unbrowsed females (70.1% vs 67.6% germination for browsed vs unbrowsed males, $\chi^2=0.162$, $df=1$, $P=0.72$; Table 1). Thus, results of the hand-pollination experiment indicated that there were no significant differences in pollen viabilities. This result was further substantiated from pollen counts and viability analyses, with no significant differences between browsed and unbrowsed plants ($t=0.786$, $df=24$, $P=0.439$ and $t=0.719$, $df=28$, $P=0.478$ for pollen counts and viability analyses, respectively; Table 1). In addition, no significant differences were found in any floral traits (MANOVA, $F=1.36$, $df=5,43$, $P=0.257$) that could differentially affect pollen transport between browsed and unbrowsed plants.

In terms of flower production, browsed plants produced 1.8 times as many flowers as plants that were not browsed at the height of pollinator activity (174.69±17.4 vs 96.79±15.9 flowers per plant for browsed and unbrowsed individuals, respectively; $F=10.73$, $df=1,52$, $P=0.002$). By the end of the flowering season there was only a non-significant trend toward higher flower production by browsed plants, with browsed plants producing, on the average, 1.43 times as many flowers as unbrowsed plants (210.45±29.7 vs 147.48±31.2 flowers per plant for browsed and unbrowsed plants; $F=2.03$, $df=1,18$, $P=0.17$). As in a previous study (Maschinski and Whitham 1989), there were no significant differ-

ences in the number of fruits produced between browsed and unbrowsed plants (133.25±16.3 vs 105.92±17.2 fruits per plant for browsed and unbrowsed individuals, respectively; $F=1.27$, $df=1,18$, $P=0.28$). There were also no significant differences in the number of seed/fruit or seed weights between browsed and unbrowsed plants, as shown in the hand-pollination experiments described above (Experiment 1, Table 1). There were, however, significant differences in germination success between browsed and unbrowsed females. Unbrowsed females had significantly higher germination success than browsed females when crossed with either browsed (70.1% vs 56.6%; $\chi^2=4.10$, $df=1$, $P<0.05$) or unbrowsed males (67.6% vs 56.1%; $\chi^2=4.26$, $df=1$, $P<0.05$, Experiment 1, Table 1). Higher germination success, however, does not appear to confer a fitness advantage to unbrowsed females. When we multiply the mean number of fruits per plant by the mean number of seeds per fruit by the mean percentage germination, browsed individuals produce an average of 571 viable seeds per plant while unbrowsed individuals produce an average of 562 viable seeds per plant. Thus, at the maternal level browsed plants appear to compensate equally.

The patterns of flower and fruit production described above appear robust given that similar patterns were also found in an earlier study. In 1987, browsed plants produced 1.97 times as many flowers as unbrowsed plants (229.06±28.35 vs 116.05±28.35 flowers per plant for browsed and unbrowsed individuals, respectively; $F=7.94$, $df=1,15$, $P=0.013$) with no significant differences in the number of fruits produced (40.66±9.30 vs 23.89±9.30 fruits per plant for browsed and unbrowsed individuals, respectively; $F=1.62$, $df=1,15$, $P=0.222$) by the end of the flowering season. Thus, even though flower production was significantly enhanced by ungulate herbivory during the peak of pollinator activity, and/or maintained throughout the entire flowering season depending upon year, maternal fitness was not. Given that pollinator activity significantly declines throughout the flowering season, we predicted that higher flower production by browsed individuals observed mid-season in the 1997 experiments would translate into higher browsed male fitness. Results of the natural pollination experiment, described below, support this prediction.

Using the above data we calculated the number of viable pollen grains produced per plant with the potential to sire offspring for both browsed and unbrowsed plants. We multiplied the mean number of flowers produced per plant (mid-season) by the mean number of pollen grains produced per flower times the mean percentage of viable pollen (Table 1). Results showed that, at the individual plant level, browsed plants produced 2.1-fold as much viable pollen mid-season as plants that were not browsed (1,452,675 vs 690,861 viable pollen grains per plant for browsed vs unbrowsed plants). The increase in pollen production by browsed plants is due to an increase in flower production given that there were no significant differences in pollen production or pollen viability per flower.

Natural pollination experiment

The natural pollination experiment and genetic analysis, which was designed to assess the paternal contribution of browsed versus unbrowsed individuals under natural conditions, confirmed that higher flower, and hence, higher pollen production by browsed plants translates into an increase in paternal fitness. Among 25 RAPD primers screened, nine were polymorphic, allowing us to either categorize to browsed or unbrowsed sires or to definitively assign the paternity of 26 of the 33 offspring reared from equal numbers of browsed and unbrowsed parents. Paternity was assigned to 2–4 offspring per maternal plant across 9 of the 10 dams; one unbrowsed maternal plant did not produce any scorable offspring. As an internal control on the reliability of the technique, we blindly assigned or categorized 20 of the 33 offspring back to known mothers or to a group of dams. In all 20 cases the offspring were correctly assigned to known mothers or categorized to browsed or unbrowsed dams giving us confidence in assigning or categorizing paternity. Among the 26 paternally assigned offspring, 18 were sired by browsed fathers and 8 were sired by unbrowsed fathers which differs significantly from a ratio of 50:50 by a binomial test, P=0.05. This represents an approximate 2.25-fold increase in paternal fitness by browsed over unbrowsed individuals. This is also in accord with the 2.1-fold higher pollen production by plants that were browsed, with no significant difference in either the ratio of browsed and unbrowsed flower production mid season to the number of browsed and unbrowsed offspring produced (χ^2=0.140, df=1, P>0.70) or the number of predicted (based on the 2.1-fold increase in pollen production) to observed browsed and unbrowsed offspring produced (χ^2=0.012, df=1, P>0.90, Table 1). Overall, these results indicate that an increase in flower and pollen production directly translates into an increase in paternal fitness. Not only was there an approximate 2-fold advantage at the individual plant level, but there were also as many browsed plants as unbrowsed plants (53% browsed or 1.13 times as many) in the population, giving browsed plants an approximate 2.37-fold advantage at the population level. Of course we are aware that this is only a single small experiment that should be repeated, perhaps several times, each with larger sample sizes. Nonetheless, these data are consistent with calculated predictions noted above and with previous paternal results from a second species of *Ipomopsis*, *I. aggregata* (Gronemeyer et al. 1997).

In conclusion, estimates of the effects of herbivory on individual plant fitness based on measures of female fitness alone may be misleading if plants either experience a reduction in male fitness or, alternatively, experience enhanced male fitness. Only a few studies have documented male and female fitness simultaneously (Strauss et al. 1996); most have shown that male and female fitness are negatively or weakly correlated (Bertin 1982; Broyles and Wyatt 1990; Devlin and Ellstrand 1990; Ennos and Dodson 1987; Schlichting and Devlin 1989; Strauss et al. 1996) and at least one has shown strong positive correlations between male and female fitness (Gronemeyer et al. 1997). Thus, an accurate picture of plant-herbivore interactions must include the paternal as well as the maternal side of fitness.

Although overcompensation has been reported for a number of plant species, in only one case has paternal fitness been assessed (Gronemeyer et al. 1997). In this particular case both male and female components of fitness were enhanced following herbivory. Here we show that herbivory can result in an overall increase in fitness due solely to an increase in paternal fitness. This is of potential widespread importance in that there are many documented cases where plant species increase flower production following apical damage but where fruit and seed production is unaffected or reduced (Hendrix 1979; Inouye 1982; Lennartsson et al. 1997; Lowenberg 1994; Paige 1994; Reichman and Smith 1991). In such cases, the paternal component of fitness could be enhanced through an increase in flower, and hence pollen, production, as in this study. Thus, overcompensation may be a more common phenomenon than presently thought. Such a finding would fundamentally broaden our present views on the ways in which plants can overcome or even take advantage of being eaten, adding to the suite of general possible outcomes.

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