

## HOST SPECIES AFFECTS HERBIVORY, POLLINATION, AND REPRODUCTION IN EXPERIMENTS WITH PARASITIC *CASTILLEJA*

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**Abstract.** The relative performance of a parasitic plant on different host species will depend on both direct and indirect effects of hosts on parasite interactions with mutualists and antagonists. Host species could affect parasite interactions with both herbivores and pollinators due to the uptake of defensive compounds and nutrients. However, the effects of different host species on parasitic plants have not been experimentally tested in the field. I determined the effect of two native host species, an alkaloid-producing, nitrogen-fixing lupine and non-alkaloid, non-nitrogen-fixing grass, on herbivory, pollination, and reproduction of the hemiparasitic plant Indian paintbrush (*Castilleja indivisa*). Within this experiment, I manipulated herbivory and pollination to determine their effects on Indian paintbrush reproduction. Indian paintbrush parasitizing lupines produced three times as many seeds and were more attractive to pollinators than Indian paintbrush parasitizing grass. However, there was no effect of host species on early season or floral herbivory. In addition, MANOVA revealed that host species influenced the response of Indian paintbrush female reproduction to experimentally manipulated herbivory and pollination treatments. Thus, the effect of hosts on parasites is mediated by interactions with herbivores and pollinators, and both direct and indirect effects may shape the selective pressures mediating interactions between hosts and parasites.

**Key words:** *Andropogon gerardii*; *Castilleja indivisa*; *diffuse coevolution*; *Indian paintbrush*; *indirect effects*; *Lupinus texensis*; *nitrogen fixation*; *parasitism*; *tritrophic interactions*.

### INTRODUCTION

Parasites are ubiquitous in nature and can affect hosts directly and indirectly. For example, parasites and herbivores can reduce host fitness directly by reducing resources available for reproduction (Marquis 1992, Poulin 1998) or the attraction of mates (Hillgarth and Wingfeld 1997), and indirectly by reducing competitive ability (Price 1980, Louda et al. 1990) or by interfering with mutualists such as pollinators (Gomez 1994, Strauss et al. 1996, Lehtila and Strauss 1997, Krupnick et al. 1999, Mothershead and Marquis 2000, Adler et al. 2001) or mycorrhizae (e.g., Gehring and Whitham 1992, Davies and Graves 1998, Salonen et al. 2001, Gange et al. 2002). Hosts can have equally complex direct and indirect effects on their parasites. While the effect of host species on herbivore performance and on tritrophic interactions has been extensively studied (reviewed in Duffey 1980, Price et al. 1980, Hare 1992), we know much less about how generalist parasites are affected by different hosts.

Parasitic plants are present in every major ecosystem (Kuijt 1969, Press and Graves 1995), can alter the outcome of competition between species (Gibson and Watkinson 1991, Matthies 1996), and have been shown in both experimental studies and theoretical work to play a major role in determining community structure (Gib-

son and Watkinson 1992, Pennings and Callaway 1996, Marvier 1998b, Smith 2000). However, the study of parasitic plant–host plant interactions has focused largely on laboratory studies and studies of crop pests, rather than studies of natural communities (Pennings and Callaway 2002). Generalist parasitic plants, like generalist herbivores, may be directly affected by the host species they parasitize. Even hemiparasitic plants, which are photosynthetic, acquire a considerable portion of their fixed carbon and nitrogen from their host plants (Press 1989, Seel et al. 1992). Increased parasite performance on certain host species may be due in part to greater availability of resources from those hosts. For example, legumes differ from nonleguminous hosts in their ability to fix nitrogen (via association with symbionts) and the greater performance of many parasitic plants on leguminous hosts is generally attributed to increased nitrogen availability (Gibson and Watkinson 1989, Seel and Press 1993, 1994, Matthies 1996, 1997, Press and Seel 1996, Marvier 1998a, Adler 2002).

In addition to direct effects, host species may also indirectly affect parasitic plant success by altering interactions between the parasite and its community of mutualists, such as pollinators, and antagonists, such as herbivores. The net effect of a host on its parasite may be difficult to predict in a community context due to the complexity of direct and indirect interactions. For example, parasitic plants often acquire secondary compounds from hosts (Arslanian et al. 1990, Boros et

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al. 1991, Mead et al. 1992, Stermitz and Pomeroy 1992, Stermitz et al. 1993, Adler and Wink 2001). The uptake of secondary compounds could increase parasite resistance to herbivores (Adler 2000) and also increase pollinator attraction by reducing herbivory (Adler et al. 2001). However, secondary compounds could also impose a cost if they are deterrent to pollinators as well as herbivores (Strauss et al. 1999). Similarly, the uptake of nitrogen from hosts could have a variety of costs and benefits. Nitrogen might make hemiparasites more palatable to their herbivores (Marvier 1995, Kyto et al. 1996), but increased nitrogen could also increase allocation to pollinator attraction (Frazee and Marquis 1994, Gardener and Gillman 2001). Thus, multifactorial experiments manipulating herbivory and pollination are needed to detect and separate direct and indirect effects.

The effect of lupine hosts compared to other non-leguminous hosts on their parasites will depend on the relative importance of nitrogen uptake, alkaloid uptake, and other differences on both parasitic plant growth and indirect effects on the animal community. If alkaloid uptake is the most important factor determining these interactions, we might expect herbivores and possibly pollinators to avoid parasitic plants parasitizing lupines. Conversely, if nitrogen has the greatest influence, pollinators and herbivores might prefer parasitic plants on lupine despite the increased alkaloid uptake. In this study, I experimentally compared the effect of the native lupine host *Lupinus texensis* or grass host *Andropogon gerardii* on herbivory, pollination, and lifetime seed production in the hemiparasite *C. indivisa*. In order to determine the impact of herbivory and pollination on seed production, I manipulated herbivory (pesticide or control) and pollination (hand-pollination or control) of *C. indivisa* parasitizing each host in a fully factorial design. To my knowledge, this is the first study to experimentally determine the effects of native host species on parasitic plant performance in the field, where community interactions may play an important role.

#### MATERIALS AND METHODS

*Castilleja indivisa* Engelman (Scrophulariaceae), or Indian paintbrush, is an annual hemiparasite endemic to Texas (Loughmiller and Loughmiller 1984). Although many species of *Castilleja* are called Indian paintbrush, this common name will be used here to refer to *C. indivisa* specifically. Indian paintbrush is self-incompatible (L. S. Adler and C. Huyghe, unpublished data) with inconspicuous flowers and brightly colored bracts. Root parasites, including Indian paintbrush, parasitize hosts by establishing connections to the host vascular system via haustoria (Kuijt 1969) and can parasitize a wide range of hosts (Sweatt 1997). Indian paintbrush do not produce alkaloids, but take up the alkaloid lupanine when parasitizing a common host, *Lupinus texensis* (Stermitz and Pomeroy 1992).

These alkaloids are produced in the chloroplasts of lupines but are transported via the phloem to all plant parts, including the roots (Wink 1992). When Indian paintbrush parasitize other common hosts, such as grasses, these alkaloids are absent (L. S. Adler, unpublished data).

*Lupinus texensis* Hook and *Andropogon gerardii* L. are both common, native plant species in central Texas. *Lupinus texensis* (Fabaceae; hereafter lupine) is an annual, endemic to Texas, that frequently grows in large fields with Indian paintbrush and flowers concurrently (Loughmiller and Loughmiller 1984). *Andropogon gerardii*, or big bluestem (Poaceae; hereafter grass) is one of the major perennial grasses of the tallgrass prairies, common in prairies and open woods throughout Texas (Hatch and Pluhar 1993). Big bluestem flowers in late summer to early fall (Hatch and Pluhar 1993). While this range grass species typically limits forb establishment when it is dense, the early flowering of Indian paintbrush before extensive grass growth may allow coexistence (S. Archer, personal communication).

Indian paintbrush frequently grow in stands as dense as in this experiment, and commonly parasitize lupines in the field. I found 8.6 plants/m<sup>2</sup> and 13.5 plants/m<sup>2</sup> in transects of two different natural populations (Stengl House Reserve in Bastrop, Texas, and the former site of the Lady Bird Johnson Wildflower Center, 2600 FM 973 North, ~4.8 km north of Highway 71). In each of these sites, 42% and 30% of plants contained lupine alkaloids. Unfortunately, it is extremely difficult to determine the rate at which Indian paintbrush parasitize grasses such as big bluestem because there are no easily detected chemical markers like the alkaloids of lupines. Digging up plants to find haustoria is time-consuming, destructive, and often misleading, as parasitic plants can form haustoria even in response to dead logs (M. Marvier, personal communication). However, grasses are a common host for many hemiparasites in the field (e.g., Marvier 1998b).

To experimentally determine the effect of host species on herbivory, pollination, and seed production of Indian paintbrush, individuals were grown from seed and randomly assigned to either lupine or grass hosts. Six lupine hosts or 15 mL of grass seed (~350 seeds) were planted with multiple Indian paintbrush per 10-cm pot in a greenhouse in January 1999. Lupine seeds were inoculated prior to planting with *Rhizobium* spp. in a milk medium. Lupine seed, grass seed, and inoculant were purchased from Native American Seed (Junction, Texas, USA) and Indian paintbrush seed were provided from natural collections by the Lady Bird Johnson Wildflower Center. Plants grew in a 1:1 ratio of University of California Davis soil mix:vermiculite (Baker 1972, Evans 1998) with a mean temperature of 18°C under a day:night regime of 16:8 h created with a 1000-W metal halide light. In March, Indian paintbrush were thinned to one parasite per pot with six lupine hosts or approximately 30 grass indi-

viduals, and transplanted to a fenced natural area in the Lady Bird Johnson Wildflower Center, Austin, Texas, USA. Each parasite-host replicate was surrounded by a buried cylinder of nonwoven polypropylene fabric (Root Control, Oklahoma City, Oklahoma, USA) 22 cm in depth with a 25 cm diameter, to allow water penetration but prevent parasitism on other wild plants. Plants were randomized within 15 blocks. Plants within a block were in two rows, separated by ~27 cm from their nearest neighbors, and blocks were separated by rows 60 cm wide. Each block contained two replicates of each host–herbivory–pollination combination (see below), for a total of 240 plants. Due to some mortality or failure to flower, 181 plants were used in analyses.

I determined the effects of host species on pollination by observing pollination for periods ranging from 1.5 to 7 h/d over 10 days for a total of 31 h. Most observations took place between 1500 and 2000 hours, the period of greatest pollinator activity that season. The bumble bee *Bombus pennsylvanicus* was the primary floral visitor (89.3% of all flowers probed; most other visits were from a variety of butterflies). I observed the entire field plot (240 plants) simultaneously, and followed each pollinator from the time it entered the field plot until it left. I used a handheld tape recorder and noted the number of visits to each plant, the number of flowers probed per visit, and the time spent per flower in seconds (determined by timing the recorded interval on tapes). All host inflorescences were clipped to prevent host floral display from affecting pollinator preference and to prevent the introduction of nonlocal plant populations at the Wildflower Center.

I measured early and late season herbivory using two methods. In an early season herbivory census on 12 April, I considered plants “damaged” if either the leaf or meristem was damaged. At the end of the flowering season in late May, the fate of every flower (filled or unfilled fruit, and damaged or undamaged) was recorded, and seeds were counted for every filled fruit. The most common leaf and meristem herbivores were larvae of the moth *Plusia biloba* (Noctuidae) and the buckeye butterfly *Junonia coenia* (Nymphalidae), and the most common floral, fruit, and seed herbivore was larvae of the moth *Endotheria hebesana* (Tortricidae).

Within this experiment, Indian paintbrush plants with each host were assigned to pesticide (spray or control) and hand-pollination (hand or control) treatments in a factorial design to determine the impact of herbivory and pollination on seed production. In the spray treatment, half of the Indian paintbrush were sprayed twice weekly with *Bacillus thuringiensis*, a biodegradable nonsystemic pesticide specific to Lepidoptera (Thuricide Concentrate, Bonham, Texas, USA) from the time they were transplanted until harvesting. Control plants were sprayed with water and host plants were not sprayed. *B. thuringiensis* in artificial diet did not affect larval survival or pupal dry mass of the honey bee *Apis mellifera* (Arpaia 1996), suggesting that pes-

ticide application is not likely to have direct effects on bee pollinators.

In the hand-pollination treatment, all Indian paintbrush were exposed to natural pollination, and half the plants received supplemental hand-pollination twice weekly to determine whether pollinators were limiting seed production. To hand-pollinate, I collected a mix of pollen from other plants in the hand-pollination treatment (to avoid transferring pollen to naturally pollinated plants) using a camelhair paintbrush and then applied it to stigmas. On 17 April and 6 May, I measured plant height in centimeters, number of inflorescences, number of open flowers per inflorescence, length of inflorescences in centimeters, and length of the longest corolla in millimeters for use as covariates in analyses of pollinator preference. I also recorded the date at which flowering began and ended for each plant to calculate the total flowering period.

The effect of host, pesticide, and hand-pollination treatment on plant female reproduction, measured as flowers, fruits, seeds, proportion of flowers setting fruit, and flowering period, was analyzed with MANOVA using the GLM procedure of SAS version 8 (SAS Institute 1999). I performed univariate tests when MANOVA analysis revealed significant results of host, pesticide, or hand-pollination as a main effect or interaction term (Scheiner 1993). All main effects were fixed and all interactions were tested except interactions with block; thus I tested the null hypothesis that there was no treatment effect in any block, as is recommended when block size is arbitrarily defined (Newman et al. 1997). All proportional data were arcsine(square root( $x$ )) transformed, and other data were  $\log(x + 1)$  transformed to meet assumptions of normality. “Flowering period” was not transformed.

Early season herbivory was measured as damaged/not damaged, a categorical variable. The effect of block and treatments on early season damage was therefore assessed using categorical modeling with PROC CATMOD of SAS (SAS Institute 1999). Floral herbivory, measured as the proportion of buds damaged at any point (buds, flowers, or fruit), was analyzed with ANOVA using the same model described previously for plant reproduction.

Pollinator preference was measured as both the number of visits to each plant (a measure of the decision to visit, a choice made before contact with the plant) and as the number of flowers probed and time per flower probe (a measure of the quality of the visit, reflecting the decision to continue foraging after probing a flower). Only bumble bee visitation was analyzed. The effect of host, pesticide, hand-pollination, and their interactions on the number of visits, number of flowers probed per visit, and time spent per flower was analyzed with ANOVA. Number of flowers probed and time spent per flower were analyzed only for the subset of plants that were visited ( $n = 68$ ). The relationship between floral traits (plant height, number of inflores-

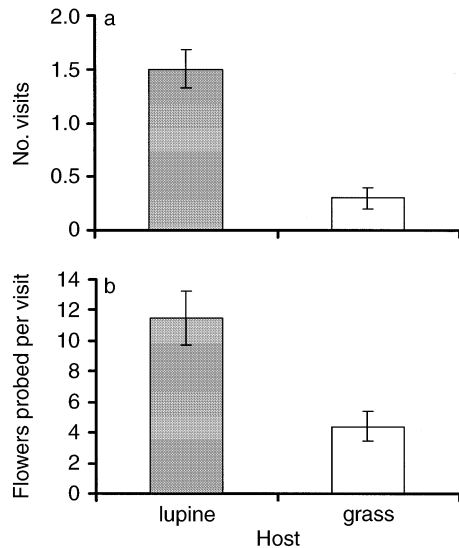


FIG. 1. The effect of host species on (a) the number of visits to *C. indivisa* plants ( $n = 105$  for lupine and 70 for grass), and (b) the number of flowers probed by pollinators per visit ( $n = 58$  for lupine and 10 for grass). Data for (b) are only for plants that were visited by pollinators (i.e., no zero values were used). Error bars represent  $\pm 1$  SE. All effects are significant at  $P < 0.01$ .

cences, number of open flowers, inflorescence length, and corolla length) and pollinator visitation was determined with multiple regression; traits that significantly influenced pollinators were subsequently incorporated into ANCOVAs to determine if the effect of host treatment on pollinator preference was due to the influence of host on these traits.

## RESULTS

### *Effect of treatments on herbivory*

The effect of pesticide manifested itself early in the season; spraying pesticides significantly reduced the proportion of plants experiencing early season damage to meristems or leaves (19.2% of sprayed plants vs. 41.6% of control plants had early season damage;  $\chi^2 = 15.53$ ,  $P < 0.0001$ ). No other factor except block affected early season damage ( $\chi^2 < 1.0$ ,  $P > 0.3$  for all). No factor had any effect on floral herbivory except for block (Appendix A); host species was only marginally significant ( $F_{1,142} = 2.95$ ,  $P = 0.09$ ) and pesticides had no effect ( $F_{1,142} = 0.08$ ,  $P > 0.35$ ).

### *Effect of treatments on pollinator preference*

Bumble bee pollinators strongly preferred Indian paintbrush parasitizing lupines to those parasitizing grasses. Indian paintbrush parasitizing lupines received significantly more visits per plant ( $F_{1,174} = 31.20$ ,  $P < 0.0001$ ) and had more flowers probed per visit than Indian paintbrush parasitizing grass ( $F_{1,46} = 8.13$ ,  $P < 0.01$ , Appendix B, Fig. 1). Hand-pollination resulted in more time spent in flowers on Indian paintbrush

parasitizing grass but not lupines ( $F_{1,45} = 4.25$ ,  $P < 0.05$ , Appendices B, C). Pesticide application did not affect any measure of pollinator preference (Appendix B).

The effect of host species on pollinator visits may be mediated by floral traits. Number of inflorescences and number of open flowers were the only variables that significantly affected pollinator preference in multiple regressions (slopes, number of visits =  $0.14 \times$  number of inflorescences,  $P < 0.0001$ ; flowers probed =  $0.15 \times$  open flowers +  $0.15 \times$  number of inflorescences,  $P = 0.023$ ,  $0.0012$ , respectively; time per flower, no significant regressions). When these variables were included as covariates in an ANCOVA, the effect of host on flowers probed disappeared ( $F_{1,44} = 1.35$ ,  $P > 0.25$ ) and was reduced but still significant for the total number of visits ( $F_{1,102} = 4.2$ ,  $P = 0.043$ ). Thus, much of the effect of host species on pollinator attraction can be explained through effects on floral display.

### *Effect of treatments on Indian paintbrush female reproduction*

MANOVA revealed that Indian paintbrush female reproduction was significantly affected by host, hand-pollination, the host  $\times$  hand-pollination interaction, and the host  $\times$  pesticide interaction (Appendix D;  $P < 0.02$  for pesticide  $\times$  host,  $P < 0.0001$  for all others). Thus, the effects of pesticides and hand-pollination on Indian paintbrush reproduction depend on host species, and there are also overall effects of host and hand-pollination. Because significant MANOVA can indicate a multivariate response to treatments that is not due to any single response variable, I present means of all MANOVA response variables for each host-pesticide-hand-pollination combination in Appendix C.

Univariate analyses were examined to determine which variables might be driving these effects. There was a strong effect of host plant on most components of reproduction; Indian paintbrush parasitizing lupines flowered eight days longer ( $F_{1,142} = 43.43$ ,  $P < 0.0001$ ), produced six times more flowers ( $F_{1,142} = 368.93$ ,  $P < 0.0001$ ), and produced three times more fruits ( $F_{1,142} = 62.10$ ,  $P < 0.0001$ ) and seeds ( $F_{1,142} = 32.97$ ,  $P < 0.0001$ ) than Indian paintbrush parasitizing grass hosts (Appendix A, Fig. 2). Indian paintbrush were also pollen-limited; hand-pollinating more than doubled the proportion of buds that set fruit and quadrupled the seeds produced ( $0.11 \pm 0.01$  compared to  $0.30 \pm 0.02$  buds set fruit,  $398 \pm 67$  compared to  $1871 \pm 257$  seeds produced,  $F_{1,142} > 32$ ,  $P < 0.0001$  for all, Appendix A).

Plants that were sprayed also had a longer flowering season ( $17.7 \pm 0.9$  d compared to  $21.0 \pm 1.1$  d,  $F_{1,142} = 4.78$ ,  $P < 0.05$ , Appendix A), due primarily to an earlier flowering time. However, host species mediated the effect of pesticides on flowering period. Pesticides increased the flowering period of Indian paintbrush parasitizing lupines but did not affect the flowering period of Indian paintbrush parasitizing grass (host  $\times$  pesti-



FIG. 2. The effect of host species on seeds, flowers, fruit, and flowering period (in days) in the hemiparasite *C. indivisa*. Error bars represent  $\pm 1$  SE. Note the different scale for seeds compared to other traits. All effects are significant at  $P < 0.0001$ .

cide,  $F_{1,142} = 8.27$ ,  $P < 0.01$ , Appendix A, Fig. 3). Although the interactions between host and both pesticide and hand-pollination treatments were significant in the MANOVA, the interaction between host and pesticide on flowering date was the only significant univariate interaction. Thus, the effect of pesticides and hand-pollination on Indian paintbrush female reproduction is dependent on host species, but this effect is due to a multidimensional response rather than any one variable alone (Scheiner 1993).

There was a significant interaction between hand-pollination and pesticide spraying on flowering period ( $F_{1,142} = 6.25$ ,  $P < 0.02$ ). However, this interaction was not significant in the MANOVA and further appears to be due to an earlier date of flowering rather than extended flowering (Appendix C). It is difficult to imagine how hand-pollination, which does not begin until plants flower, could affect the date of first flowering. The most parsimonious explanation is that, although plants were randomly assigned to treatments, plants assigned to the control herbivory and pollination treatment happened to flower later; even randomization cannot prevent occasional unwanted biases between

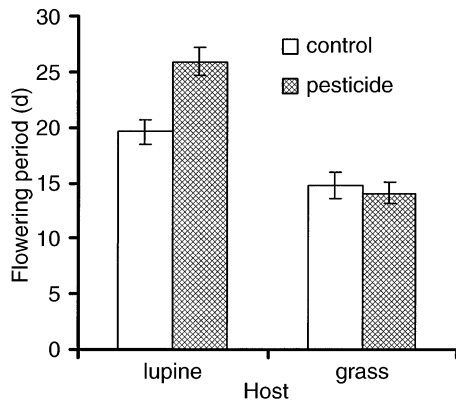


FIG. 3. Interaction between host species and pesticide treatment on total flowering period. Error bars represent  $\pm 1$  SE.

treatments (Hurlbert 1984) and this bias did not affect any subsequent measure of plant reproduction.

DISCUSSION

Host species had a considerable effect on lifetime seed production of their parasites; Indian paintbrush parasitizing lupines had longer flowering periods, produced six times more flowers and produced three times more fruits and seeds. There are a variety of direct and indirect mechanisms by which lupine hosts could benefit their parasites more than grass hosts. The uptake of alkaloids has been shown to reduce herbivory in Indian paintbrush (Adler 2000) and also has potential costs in terms of autotoxicity (L. S. Adler and C. Huyghe, unpublished data). However, host species did not affect herbivory in this study, suggesting that this potential indirect effect did not play a significant role in improving parasite reproduction. Increased nitrogen availability may be responsible for the great increase in seed production. Several other studies have found that hemiparasites are most successful on leguminous hosts compared to nonlegumes or grasses (Gibson and Watkinson 1989, Seel and Press 1993, 1994, Matthies 1996, 1997, Press and Seel 1996, Marvier 1998a, Adler 2002), suggesting that hemiparasites may be nitrogen-limited. In addition to this direct effect, resources from lupine hosts may be responsible for strong differences in pollinator attraction via increased floral display (Fig. 1). Indian paintbrush were pollen-limited, and thus increased pollinator visits may contribute substantially to plant female fitness. Changes in pollinator preference often affect male reproduction as much or more than female reproduction (Stanton et al. 1986, Young and Stanton 1990) and thus, attracting pollinators may increase male as well as female reproduction.

Aside from providing resources or defensive compounds, host species may affect their plant parasites via competition or shared interactions. Parasitic plants can be thought of as intraguild predators, in that they feed on other species in the same trophic level (Polis and Holt 1992). Hemiparasites therefore compete with

their hosts for light and soil resources (Matthies 1995). Big bluestem grass typically forms tall, dense stands and may shade out forbs (Hatch and Pluhar 1993), while Texas lupine is most common in disturbed sites and is thought to be a poor competitor (Loughmiller and Loughmiller 1984). Thus, some of the benefit of parasitizing lupines may be due to reduced competition. Parasites and hosts also have the potential to share interactions that may be beneficial or detrimental. For example, junipers that host mistletoe parasites have increased fruit dispersal due to a shared frugivore (van Ommeren and Whitham 2002), and hosts of parasitic dodder may experience increased herbivory due to a dodder weevil that also feeds on host plants (Anderson 1970). A similar mechanism is possible between Indian paintbrush and lupines because bumble bees were the primary pollinators of both species; increased floral display could serve to increase pollinator visitation to both species or reduce visitation to one species via competition (Waser 1978, Campbell 1985, Kohn and Waser 1985). Although this interaction did not occur in the current study because host flowers were clipped, future studies should address the possibility of this additional host–parasite interaction.

Both nutrient and defensive compound uptake are likely to influence herbivore preference nonlinearly (Slansky and Wheeler 1992, Behmer et al. 2002, Vilalba et al. 2002). Host species did not affect herbivory on Indian paintbrush in this study, despite strong effects of alkaloid uptake on Indian paintbrush herbivory at the same site in the previous year (Adler 2000). For parasitic plants, the combined effects on herbivore preference of increased nitrogen concentration (Adler 2002) and increased defensive compounds (Stermitz and Pomeroy 1992, Adler and Wink 2001) from lupine hosts may depend on the herbivore. Other studies have found increased herbivory, decreased herbivory, or no effect when comparing plants parasitizing lupines to plants parasitizing other hosts (Stermitz et al. 1989, Marvier 1996, Adler 2002). However, a parasitic plant obtaining alkaloids from a nonlegume host had reduced herbivore preference compared to parasites on all other hosts (Marko 1996), suggesting that alkaloid uptake is detrimental to herbivores in the absence of concurrent nitrogen uptake.

A central question in evolutionary ecology is how species coevolve. Pairwise evolution implies that the coevolutionary dynamics of two species can be understood without considering additional species, while diffuse coevolution implies that coevolutionary dynamics are influenced by the presence of additional species (Janzen 1980, Fox 1981, Stinchcombe and Rausher 2001). Criteria for determining whether two species undergo pairwise or diffuse selection have been defined (Hougen-Eitzman and Rausher 1994, Iwao and Rausher 1997, Stinchcombe and Rausher 2001) and include determining whether the pattern of natural selection does not change (pairwise) or changes (diffuse)

in the presence of other species (Stinchcombe and Rausher 2001). In the current study, the effect of both experimentally increased pollination and reduced herbivory on parasitic plant reproduction depended on host species (Appendix D). This result suggests that the magnitude or direction of selection by both pollinators and herbivores on Indian paintbrush may depend on the host species. Thus, coevolution between hosts and parasites in this system may be diffuse rather than pairwise.

A discussion of diffuse vs. pairwise coevolution assumes that generalist parasitic plants can coevolve with hosts. While some studies have documented host preference in generalist parasitic plants (Werth and Riopel 1979, Gibson and Watkinson 1989, Kelly 1990, 1992, Nilsson and Svensson 1997, Yoder 1997, Norton and De Lange 1999), for host preference to evolve in response to selection there must be heritable variation in this trait (Falconer 1989). Models suggest that unpredictable host environments, such as the high-disturbance, annual-dominated meadows where *C. indivisa* is abundant, favor parasite generalization over specialization (Lapchin 2002). Recent studies have found only limited support for local adaptation of parasites to host populations, measured by parasite performance (Lammi et al. 1999, Koskela et al. 2000, Mutikainen et al. 2000), and evidence for local adaptation to hosts is weak in generalist compared to specialist parasites (Lajeunesse and Forbes 2002). Intraspecific variation in host preference has only been examined in one study to my knowledge. Maternal sibships of the annual hemiparasite *Triphysaria pusilla* varied in their preference for a lupine host (*L. nanus*) compared to a grass host (*Bromus carinatus*) (L. S. Adler and J. I. Yoder, unpublished manuscript), and plants whose maternal host was lupine showed a stronger preference for lupine than plants with other maternal hosts. However, when we grew plants without hosts and assayed their offspring, host preference disappeared, suggesting that preference is determined more by maternal host than by genetic variation. Since host preference appears to be determined more by maternal host environment rather than genetic factors, specialization may be unlikely to evolve.

Studies have shown that hemiparasites, by having the strongest impacts on the best competitors, can alter the outcome of competition between species (Gibson and Watkinson 1991, Matthies 1996) and structure communities (Gibson and Watkinson 1992, Pennings and Callaway 1996, Marvier 1998b, Smith 2000). The current study has shown that hosts also affect their parasites, and that this effect is mediated in part by the community of herbivores and pollinators. Thus, we must consider both the qualities of different host species and the community context of interactions in order to define the selective pressures mediating interactions between parasites and their hosts.

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## LITERATURE CITED

- Adler, L. S. 2000. Alkaloid uptake increases fitness in a hemiparasitic plant via reduced herbivory and increased pollination. *American Naturalist* **156**:92–99.
- Adler, L. S. 2002. Host effects on herbivory and pollination in a hemiparasitic plant. *Ecology* **83**:2700–2710.
- Adler, L. S., R. Karban, and S. Y. Strauss. 2001. Direct and indirect effects of alkaloids on plant fitness via herbivory and pollination. *Ecology* **82**:2032–2044.
- Adler, L. S., and M. Wink. 2001. Transfer of alkaloids from hosts to hemiparasites in two *Castilleja*–*Lupinus* associations: analysis of floral and vegetative tissues. *Biochemical Systematics and Ecology* **29**:551–561.
- Anderson, D. M. 1970. Dodder weevils in simultaneous association with parasitic plants and their hosts. *Science* **168**:132–133.
- Arpaia, S. 1996. Ecological impact of Bt-transgenic plants: 1. Assessing possible effects of CryIIIb toxin on honey bee (*Apis mellifera* L.) colonies. *Journal of Genetics and Breeding* **50**:315–319.
- Arslanian, R. L., G. H. Harris, and F. R. Stermitz. 1990. New quinolizidine alkaloids from *Lupinus argenteus* and its hosted root parasite *Castilleja sulphurea*: stereochemistry and conformation of some naturally occurring cyclic carbinolamides. *Journal of Organic Chemistry* **55**:1204–1210.
- Baker, F. K. 1972. The U.C. system for producing healthy container-grown plants. University of California, College of Agriculture, Davis, California, USA.
- Behmer, S. T., S. J. Simpson, and D. Raubenheimer. 2002. Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* **83**:2489–2501.
- Boros, C. A., D. R. Marshall, C. R. Caterino, and F. R. Stermitz. 1991. Iridoid and phenylpropanoid glycosides from *Orthocarpus* spp.: alkaloid content as a consequence of parasitism on *Lupinus*. *Journal of Natural Products* (Lloydia) **54**:506–513.
- Campbell, D. R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. *Ecology* **66**:544–553.
- Davies, D. M., and J. D. Graves. 1998. Interactions between arbuscular mycorrhizal fungi and the hemiparasitic angiosperm *Rhinanthus minor* during co-infection of a host. *New Phytologist* **139**:555–563.
- Duffey, S. S. 1980. Sequestration of plant natural products by insects. *Annual Review of Entomology* **25**:447–477.
- Evans, R. W. 1998. The development and properties of container soils—making a good mix, growing points. Department of Environmental Horticulture, University of California, Davis, California, USA.
- Falconer, D. S. 1989. Introduction to quantitative genetics. Third edition. Longman Scientific and Technical, New York, New York, USA.
- Fox, L. R. 1981. Defense and dynamics in plant–herbivore systems. *American Zoologist* **21**:853–864.
- Frazee, J. E., and R. J. Marquis. 1994. Environmental contribution to floral trait variation in *Chamaecrista fasciculata* (Fabaceae: Caesalpinioideae). *American Journal of Botany* **81**:206–215.
- Gange, A. C., E. Bower, and V. K. Brown. 2002. Differential effects of insect herbivory on arbuscular mycorrhizal colonization. *Oecologia* **131**:103–112.
- Gardener, M. C., and M. P. Gillman. 2001. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, *Agrostemma githago* (Caryophyllaceae). *Oikos* **92**:101–106.
- Gehring, C. A., and T. G. Whitham. 1992. Reduced mycorrhizae on *Juniperus monosperma* with mistletoe: the influence of environmental stress and tree gender on a plant parasite and plant–fungal mutualism. *Oecologia* **89**:298–303.
- Gibson, C. C., and A. R. Watkinson. 1989. The host range and selectivity of a parasitic plant: *Rhinanthus minor* L. *Oecologia* **78**:401–406.
- Gibson, C. C., and A. R. Watkinson. 1991. Host selectivity and the mediation of competition by the root hemiparasite *Rhinanthus minor*. *Oecologia* **86**:81–87.
- Gibson, C. C., and A. R. Watkinson. 1992. The role of the hemiparasitic annual *Rhinanthus minor* in determining grassland community structure. *Oecologia* **89**:62–68.
- Gomez, J. M. 1994. Importance of direct and indirect effects in the interaction between a parasitic angiosperm (*Cuscuta epithymum*) and its host plant (*Hormathophylla spinosa*). *Oikos* **71**:97–106.
- Hare, J. D. 1992. Effects of plant variation on herbivore–natural enemy interactions. Pages 278–298 in R. S. Fritz and E. L. Simms, editors. Plant resistance to herbivores and pathogens: ecology, evolution and genetics. University of Chicago Press, Chicago, Illinois, USA.
- Hatch, S. L., and J. Pluhar. 1993. Texas range plants. Texas A&M University Press, College Station, Texas, USA.
- Hillgarth, N., and J. C. Wingfeld. 1997. Testosterone and immunosuppression in vertebrates; implications for parasite-mediated sexual selection. Pages 143–155 in N. E. Beckage, editor. Parasites and pathogens: effects on host hormones and behavior. Chapman and Hall, New York, New York, USA.
- Hougen-Eitzman, D., and M. D. Rausher. 1994. Interactions between herbivorous insects and plant–insect coevolution. *American Naturalist* **143**:677–697.
- Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**:187–211.
- Iwao, K., and M. D. Rausher. 1997. Evolution of plant resistance to multiple herbivores: quantifying diffuse coevolution. *American Naturalist* **149**:316–335.
- Janzen, D. H. 1980. When is it coevolution? *Evolution* **34**:611–612.
- Kelly, C. K. 1990. Plant foraging: a marginal value model and coiling response in *Cuscuta subinclusa*. *Ecology* **71**:1916–1925.
- Kelly, C. K. 1992. Resource choice in *Cuscuta europaea*. *Proceedings of the National Academy of Sciences (USA)* **89**:12194–12197.
- Kohn, J. R., and N. M. Waser. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *American Journal of Botany* **72**:1144–1148.
- Koskela, T., V. Salonen, and P. Mutikainen. 2000. Local adaptation of a holoparasitic plant, *Cuscuta europaea*: vari-

- ation among populations. *Journal of Evolutionary Biology* **13**:749–755.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**:125–134.
- Kuijt, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, California, USA.
- Kyto, M., P. Niemela, and S. Larsson. 1996. Insects on trees: population and individual response to fertilization. *Oikos* **75**:148–159.
- Lajeunesse, M. J., and M. R. Forbes. 2002. Host range and local parasite adaptation. *Proceedings of the Royal Society of London B* **269**:703–710.
- Lammi, A., P. Siikamaki, and V. Salonen. 1999. The role of local adaptation in the relationship between an endangered root hemiparasite *Euphrasia rostkoviana*, and its host, *Agrostis capillaris*. *Ecography* **22**:145–152.
- Lapchin, L. 2002. Host–parasitoid association and diffuse coevolution: when to be a generalist? *American Naturalist* **160**:245–254.
- Lehtila, K., and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. *Oecologia* **111**:396–403.
- Louda, S. M., K. H. Keeler, and R. D. Holt. 1990. Herbivore influences on plant performance and competitive interactions. Pages 413–444 in J. B. Grace and D. Tilman, editors. *Perspectives on plant competition*. Academic Press, San Diego, California, USA.
- Loughmiller, C., and L. Loughmiller. 1984. *Texas wildflowers: a field guide*. University of Austin Press, Austin, Texas, USA.
- Marko, M. D. 1996. Hemiparasitism by *Castilleja sulphurea*: alkaloid incorporation and herbivore response. Thesis. Colorado State University, Fort Collins, Colorado, USA.
- Marquis, R. 1992. Selective impact of herbivores. Pages 300–325 in R. Fritz and E. Simms, editors. *Plant resistance to herbivores and pathogens: ecology, evolution and genetics*. University of Chicago Press, Chicago, Illinois, USA.
- Marvier, M. A. 1995. Host use by parasitic plants: effects on insect herbivore performance in a coastal plant community. *Bulletin of the Ecological Society of America* **76**:361–362.
- Marvier, M. A. 1996. Parasitic plant–host interactions: plant performance and indirect effects on parasite-feeding herbivores. *Ecology* **77**:1398–1409.
- Marvier, M. 1998a. A mixed diet improves performance and herbivore resistance of a parasitic plant. *Ecology* **79**:1272–1280.
- Marvier, M. A. 1998b. Parasite impacts on host communities: plant parasitism in a California coastal prairie. *Ecology* **79**:2616–2623.
- Matthies, D. 1995. Parasitic and competitive interactions between the hemiparasites *Rhinanthus serotinus* and *Odonites rubra* and their host *Medicago sativa*. *Journal of Ecology* **83**:245–251.
- Matthies, D. 1996. Interactions between the root hemiparasite *Melampyrum arvense* and mixtures of host plants: heterotrophic benefit and parasite-mediated competition. *Oikos* **75**:118–124.
- Matthies, D. 1997. Parasite–host interactions in *Castilleja* and *Orthocarpus*. *Canadian Journal of Botany* **75**:1252–1260.
- Mead, E. W., M. Looker, D. R. Gardner, and F. R. Stermitz. 1992. Pyrrolizidine alkaloids of *Liatris punctata* and its root parasite, *Castilleja integra*. *Phytochemistry* **31**:3255–3257.
- Mothershead, K., and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology* **81**:30–40.
- Mutikainen, P., V. Salonen, S. Puustinen, and T. Koskela. 2000. Local adaptation, resistance, and virulence in a hemiparasitic plant–host plant interaction. *Evolution* **54**:433–440.
- Newman, J. A., J. Bergelson, and A. Grafen. 1997. Blocking factors and hypothesis tests in ecology: is your statistics text wrong? *Ecology* **78**:1312–1320.
- Nilsson, C. H., and B. M. Svensson. 1997. Host affiliation in two subarctic hemiparasitic plants: *Bartsia alpina* and *Pedicularis lapponica*. *Ecoscience* **4**:80–85.
- Norton, D. A., and P. J. De Lange. 1999. Host specificity in parasitic mistletoes (Loranthaceae) in New Zealand. *Functional Ecology* **13**:552–559.
- Pennings, S. C., and R. M. Callaway. 1996. Impact of a parasitic plant on the structure and dynamics of salt marsh vegetation. *Ecology* **77**:1410–1419.
- Pennings, S. C., and R. M. Callaway. 2002. Parasitic plants: parallels and contrasts with herbivores. *Oecologia* **131**:479–489.
- Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. *Trends in Ecology and Evolution* **7**:151–154.
- Poulin, R. 1998. *Evolutionary ecology of parasites: from individuals to communities*. Chapman and Hall, London, UK.
- Press, M. C. 1989. Autotrophy and heterotrophy in root hemiparasites. *Trends in Ecology and Evolution* **4**:258–263.
- Press, M. C., and J. D. Graves. 1995. *Parasitic plants*. Chapman and Hall, London, UK.
- Press, M. C., and W. E. Seel. 1996. Interactions between hemiparasitic angiosperms and their hosts in the subarctic. Pages 151–158 in P. S. Karlsson and T. V. Callaghan, editors. *Ecological Bulletins, Number 45. Plant ecology in the subarctic Swedish Lapland*. Munksgaard, Copenhagen, Denmark.
- Price, P. W. 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton, New Jersey, USA.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* **11**:41–65.
- Salonen, V., M. Vestberg, and M. Vauhkonen. 2001. The effect of host mycorrhizal status on host plant–parasitic plant interactions. *Mycorrhiza* **11**:95–100.
- SAS Institute. 1999. *SAS/STAT user's guide, release 8 edition*. SAS Institute, Cary, North Carolina, USA.
- Scheiner, S. M. 1993. MANOVA: multiple response variables and multispecies interactions. Pages 94–112 in S. M. Scheiner and J. Gurevitch, editors. *Design and analysis of ecological experiments*. Chapman and Hall, New York, New York, USA.
- Seel, W. E., I. Cechin, C. A. Vincent, and M. C. Press. 1992. Carbon partitioning and transport in parasitic angiosperms and their hosts. Pages 199–223 in C. J. Pollock, J. F. Farrar, and A. J. Gordon, editors. *Carbon partitioning within and between organisms*. BIOS Scientific, Oxford, UK.
- Seel, W. E., and M. C. Press. 1993. Influence of the host on three sub-Arctic annual facultative root hemiparasites: I. growth, mineral accumulation and above-ground dry-matter partitioning. *New Phytologist* **125**:131–138.
- Seel, W. E., and M. C. Press. 1994. Influence of the host on three sub-Arctic annual facultative root hemiparasites: II. gas exchange characteristics and resource use-efficiency. *New Phytologist* **127**:37–44.
- Slansky, F., and G. S. Wheeler. 1992. Caterpillars compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Entomologia Experimentalis et Applicata* **65**:171–186.
- Smith, D. 2000. The population dynamics and community ecology of root hemiparasitic plants. *American Naturalist* **155**:13–23.



- Stanton, M. L., A. A. Snow, and S. N. Handel. 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science* **232**:1625–1627.
- Stermitz, F. R., G. N. Belofsky, D. Ng, and M. C. Singer. 1989. Quinolizidine alkaloids obtained by *Pedicularis semibarbata* (Scrophulariaceae) from *Lupinus fulcratus* (Leguminosae) fail to influence the specialist herbivore *Euphydryas editha* (Lepidoptera). *Journal of Chemical Ecology* **15**:2521–2530.
- Stermitz, F. R., T. A. Foderaro, and Y. X. Li. 1993. Iridoid glycoside uptake by *Castilleja integra* via root parasitism on *Penstemon teucroides*. *Phytochemistry* **32**:1151–1153.
- Stermitz, F. R., and M. Pomeroy. 1992. Iridoid glycosides from *Castilleja purpurea* and *C. indivisa*, and quinolizidine alkaloid transfer from *Lupinus texensis* to *C. indivisa* via root parasitism. *Biochemical Systematics and Ecology* **20**:473–475.
- Stinchcombe, J. R., and M. D. Rausher. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. *American Naturalist* **158**:376–388.
- Strauss, S. Y., J. K. Conner, and S. L. Rush. 1996. Foliar herbivory affects floral characters and plant attractiveness to pollinators: implications for male and female plant fitness. *American Naturalist* **147**:1098–1107.
- Strauss, S. Y., D. H. Siemens, M. B. Decher, and T. Mitchell-Olds. 1999. Ecological costs of plant resistance to herbivores in the currency of pollination. *Evolution* **53**:1105–1113.
- Sweatt, M. R. 1997. The effects of various host plants on growth, water relations, and carbon balance of the hemiparasite *Castilleja indivisa* (Indian paintbrush). Dissertation. Texas A&M University, College Station, Texas, USA.
- van Ommeren, R. J., and T. G. Whitham. 2002. Changes in interactions between juniper and mistletoe mediated by shared avian frugivores: parasitism to potential mutualism. *Oecologia* **130**:281–288.
- Villalba, J. J., F. D. Provenza, and J. P. Bryant. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos* **97**:282–292.
- Waser, N. M. 1978. Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia* **36**:223–236.
- Werth, C. R., and J. L. Riopel. 1979. A study of the host range of *Aureolaria pedicularia* (L.) Raf. (Scrophulariaceae). *American Midland Naturalist* **102**:300–306.
- Wink, M. 1992. The role of quinolizidine alkaloids in plant–insect interactions. Pages 133–169 in E. Bernays, editor. *Insect–plant interactions*. CRC Press, Boca Raton, Florida, USA.
- Yoder, J. I. 1997. A species-specific recognition system directs haustorium development in the parasitic plant *Triphysaria* (Scrophulariaceae). *Planta* **202**:407–413.
- Young, H. J., and M. L. Stanton. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* **71**:536–547.

#### APPENDIX A

A table showing ANOVAs for the effect of host species, pesticide, and hand pollination on reproduction and herbivory in Indian paintbrush is available in ESA's Electronic Data Archive: *Ecological Archives* E084-049-A1.

#### APPENDIX B

A table showing ANOVAs for the effect of host species, pesticide, and hand pollination on pollinator preference for Indian paintbrush is available in ESA's Electronic Data Archive: *Ecological Archives* E084-049-A2.

#### APPENDIX C

A table showing response variables for each treatment combination is available in ESA's Electronic Data Archive: *Ecological Archives* E084-049-A3.

#### APPENDIX D

A table showing MANOVA for effects of host species, pesticide, and hand pollination on Indian paintbrush reproduction is available in ESA's Electronic Data Archive: *Ecological Archives* E084-049-A4.