

Direct and indirect transgenerational effects alter plant-herbivore interactions

Casey P. terHorst · Jennifer A. Lau

Received: 14 September 2011 / Accepted: 8 February 2012
© Springer Science+Business Media B.V. 2012

Abstract Theory suggests that environmental effects with transgenerational consequences, including rapid evolution and maternal effects, may affect the outcome of ecological interactions. However, indirect effects occur when interactions between two species are altered by the presence of a third species, and can make the consequences of transgenerational effects difficult to predict. We manipulated the presence of insect herbivores and the competitor *Medicago polymorpha* in replicated *Lotus wrangelianus* populations. After one generation, we used seeds from the surviving *Lotus* to initiate a reciprocal transplant experiment to measure how transgenerational effects altered ecological interactions between *Lotus*, *Medicago*, and insect herbivores. Herbivore leaf damage and *Lotus* fecundity were dependent on both parental and offspring environmental conditions. The presence of insect herbivores and *Medicago* in the parental environment resulted in transgenerational changes in herbivore resistance, but these effects were non-additive, likely as a result of indirect effects in the parental environment. Indirect transgenerational effects interacted with more immediate ecological indirect effects to affect *Lotus* fecundity. These results suggest that explanations of ecological patterns require an understanding of transgenerational effects and that these effects may be difficult to predict in species-rich, natural communities where indirect effects are prevalent.

Keywords Diffuse evolution · Invasive species · Maternal effects · Non-additive interactions · Rapid evolution · Resistance

C. P. terHorst (✉) · J. A. Lau
W. K. Kellogg Biological Station, Michigan State University,
3700 East Gull Lake Drive, Hickory Corners, MI 49060, USA
e-mail: casey.terhorst@kbs.msu.edu

C. P. terHorst · J. A. Lau
Department of Plant Biology, Michigan State University, Hickory Corners,
MI 49060, USA

Introduction

Ecological experiments manipulating species interactions, such as predation, competition, or mutualism, may immediately affect population growth rates or cause changes in plastic traits. For ecological experiments that span multiple generations, however, transgenerational effects, in which parental environmental conditions affect the traits of offspring, can either increase (Bezemer et al. 1998; Reale et al. 2003) or decrease (Huxman et al. 1998, 2001; Agrawal et al. 1999; Lau et al. 2008) immediate ecological effects of the experimental treatment (reviewed in Rossiter 1996; Strauss et al. 2008). Transgenerational effects can occur across as little as one generation, as a result of either maternal effects or evolved genetic changes in traits (Rossiter 1996).

Maternal effects occur when the environment experienced by the parent affects the traits or performance of offspring (Roach and Wulff 1987). Parents in “good” environments (e.g. high resource levels) are able to better provision offspring and often produce larger, higher-quality offspring that ultimately grow faster than offspring produced by parents in “poor” environments (Rossiter 1996). Additionally, parents may supply mRNA or proteins that alter the expression of particular genes in the offspring, resulting in epigenetic effects that can affect adaptation (Youngson and Whitelaw 2008). Such transgenerational maternal effects can ultimately affect the outcomes of species interactions. For example, annual plants grown in competition with heterospecifics produced smaller seeds than plants grown alone; smaller seeds were associated with decreased competitive ability in the offspring (Platenkamp and Shaw 1993). In this case, the transgenerational effect increased the negative fitness consequences of competition. Alternately, parents growing in the presence of natural enemies can increase defenses in offspring, either by directly supplying defensive chemicals or inducing the expression of defensive proteins. This increase in defenses can result in offspring that perform better when herbivores are abundant; in other words, maternal effects can decrease the interaction strength. Both wild radish plants grown with herbivores and *Daphnia* clones grown in the presence of predators produced offspring that were more resistant to herbivory/predation because induced defenses in the parents persisted in the offspring (Agrawal et al. 1999). In these cases, the transgenerational maternal effect reduced the negative fitness consequences of herbivory and predation.

Rapid evolution can also cause transgenerational effects that alter the outcome of species interactions. Although evolutionary and ecological processes have been assumed to occur on different time scales (Slobodkin 1961), recent studies have demonstrated convincingly that evolutionary changes can be rapid and occur in one to few generations (Thompson 1998; Meyer et al. 2006; Johnson and Stinchcombe 2007; terHorst et al. 2010). As with maternal effects, these rapid evolutionary changes can alter ecological measurements of species interactions (Meyer et al. 2006; Strauss et al. 2008; terHorst et al. 2010; Ellner et al. 2011). For example, a series of theoretical and chemostat studies demonstrated that predation by rotifers selected for algal genotypes that were less susceptible to predation, resulting in the evolution of algal populations that masked the ecological effect of predation (Meyer et al. 2006; Yoshida et al. 2003, 2007). Similarly, terHorst et al. (2010) observed that, within a single predator generation (but several prey generations), the evolution of traits in protozoa reduced the ecological effect of mosquito predation on protozoa abundance.

Predictions of how transgenerational effects will alter ecological outcomes are more complex when one moves beyond pair-wise species interactions and considers multiple interacting species in diverse communities. For example, when evolution occurs in

response to more than one species, evolution can be diffuse or non-additive because of indirect effects between species (Strauss and Irwin 2004). Indirect effects occur when interactions between two species are altered by the presence of a third species. Indirect effects are particularly interesting because they increase exponentially with increasing diversity (Abrams 1992) and have the potential to swamp direct species interactions (Stone and Roberts 1991; Miller 1994). In multispecies communities, transgenerational effects may result from multiple direct species interactions, as well as indirect effects (Miller and Travis 1996). Consequently, the extent to, or manner in which, transgenerational effects alter the outcome of species interactions may depend on the diversity of the community and the resultant indirect effects among species.

The frequency and importance of ecological indirect effects have been well described (reviewed in Wootton 1994; Miller and terHorst 2011) and recent empirical work has demonstrated the potential for indirect effects to have transgenerational consequences (hereafter “indirect transgenerational effects”) (Irwin 2006; Lau 2008; terHorst 2010; Walsh and Reznick 2010). For example, decreased cell size evolved in populations of protozoa, in response to both mosquito larvae predators (terHorst et al. 2010) and protozoan competitors (terHorst 2011), but when both predators and competitors were present, protozoan cell size did not evolve because indirect transgenerational effects canceled out the direct evolutionary effects (terHorst 2010). Given the large number of indirect interactions in natural communities and the large magnitude of ecological indirect effects, indirect transgenerational effects may be common. However, the consequences of indirect transgenerational effects for contemporary ecological processes rarely have been examined (but see Bassar et al. 2010).

Here we examine how transgenerational effects resulting from experimental manipulations imposed on parental generations affect the strength of more immediate ecological interactions and fitness of a focal species, *Lotus wrangelianus*. Specifically, we ask: (1) Does parental environment affect the amount of herbivory experienced or the fitness of *L. wrangelianus* in the next generation? (2) Do indirect effects alter the effect of parental environment?

Methods

Study system

Lotus wrangelianus (Fabaceae, hereafter “*Lotus*”) is a small, primarily-selfing annual plant that inhabits open grasslands in the California Coastal Range. The exotic plant *Medicago polymorpha* (Fabaceae, hereafter “*Medicago*”) invaded California from the Mediterranean region in the late 1800s (de Haan and Barnes 1998) and now reaches high densities in many *Lotus* populations. Both *Medicago* and *Lotus* germinate with the first rains in autumn and flower and set seed in late spring. Although several insect herbivores feed on the native *Lotus*, the dominant herbivore is the exotic Egyptian alfalfa weevil (*Hypera brunneipennis*), which consumes both *Lotus* and *Medicago* leaves. *Medicago* has direct competitive effects on *Lotus*, but also has negative indirect effects on *Lotus* by increasing densities of *Hypera* (Lau and Strauss 2005). Insect herbivores increased selection on plant defenses (herbivore tolerance and resistance) in the experimental *Lotus* population growing in the selection experiment described below (Lau 2008). Insects imposed no significant selection on *Lotus* herbivore tolerance in the absence of *Medicago*, but selection for tolerance increased significantly when both insects and *Medicago* were present (Lau 2008). In

contrast, increasing *Hypera* abundance increased selection on resistance when *Medicago* was absent but not when *Medicago* was present (Lau 2008).

Selection experiment

From November 2003 through May 2004, we conducted a “natural selection in a controlled environment” experiment (sensu Conner 2003). This approach involves manipulating the environment, allowing populations to evolve for one or more generations, and then comparing offspring from the populations inhabiting different treatments. If treatments are randomly applied to replicated populations, then divergence among populations inhabiting different treatments can be definitively attributed to differential transgenerational effects (either evolutionary change or maternal effects) of the experimentally-manipulated environmental conditions. We manipulated the presence of *Medicago* and the abundance of insect herbivores in a 2×2 factorial design. Treatments were applied to 24 $3 \text{ m} \times 3 \text{ m}$ plots (6 plots per insecticide \times *Medicago* removal treatment) in one *Lotus* population that was heavily invaded by *Medicago*. The presence of *Medicago* was manipulated by removing all *Medicago* seedlings by hand in December and January after both *Medicago* and *Lotus* had germinated. Insect herbivore abundance was manipulated by applying the generalist insecticide Sevin (Bayer CropScience, RTP, North Carolina, USA) to the insecticide treatment plots and an equal amount of water to the no insecticide control plots. Insecticide treatments were applied at 2–4 week intervals depending on rainfall. Both the *Medicago* removal and insecticide treatments had minimal effects on non-target plant species (Lau and Strauss 2005), and because both *Medicago* and *Lotus* are primarily-selfing, non-target effects of insecticide on pollination were likely minimal. Furthermore, insecticide had no direct effect on *Lotus* growth in growth chamber environments where herbivores were absent (Lau and Strauss 2005). In previous work, the *Medicago* removal treatment effectively reduced *Medicago* biomass by 96% and the insecticide treatment reduced herbivory on *Lotus* by 58% (Lau and Strauss 2005).

In December 2003, 30 *Lotus* seedlings growing in the center 1 m^2 area of each treatment plot were marked. All seeds from each surviving marked individual were collected at the end of the growing season in 2004 as fruits ripened and began to dehisce. Because survival varied across plots, this resulted in seed collections from 1 to 23 maternal families per plot (mean = 13.13 SD = 5.03 families per plot). These seeds were used in a reciprocal transplant experiment in 2004–2005.

Reciprocal transplant experiment

A reciprocal transplant experiment was used to partition the effects of parental environment from more immediate ecological effects in the offspring environment (Ellner et al. 2011). *Lotus* seeds from the selection experiment were scarified and germinated in cotton plugs in tissue culture trays in a controlled environment growth chamber set to mimic field conditions. In November 2004, *Lotus* seedlings from all source plots (hereafter “parental plots”) within each parental treatment were pooled and randomly distributed among recipient treatment plots (hereafter “offspring plots”) in the same experimental population used in the selection experiment. *Medicago* removal and insecticide treatments were applied in a 2×2 factorial design to 24 $1 \text{ m} \times 1 \text{ m}$ offspring plots (6 plots per *Medicago* removal \times insecticide treatment), as above. Each of the four offspring treatments received 92 seedlings per parental treatment that were randomly distributed among the offspring plots, with the exception of the control (*Medicago* present, no insecticide) parental

treatment; only 82 seedlings from the control treatment were planted into the four offspring treatments because this parental treatment was seed limited (initial $N = 1,432$ seedlings; $n = 58$ – 60 seedlings per offspring plot). Many seedlings died as a result of the transplant process and were not included in the survival analysis described below. Additionally, a lack of survivors from each parental treatment resulted in our eliminating one replicate plot from each offspring treatment ($n = 5$ offspring plots per treatment). The surviving transplants resulted in a total of 457 seedlings ($n = 9$ – 24 families per offspring plot; $n = 8$ – 40 seedlings per offspring plot; mean seedlings per parental treatment in each offspring plot = 5.7). In May 2005, near the end of the growing season, we counted the number of leaves on the surviving seedlings and the number of leaves damaged by *Hypera*. The number of buds, the number of flowers and the number of fruits that had been initiated were also counted in one day; the sum of these was used as a measure of fecundity and an estimate of fitness.

Statistical analyses

A generalized linear mixed model was used to test the effect of insecticide and *Medicago* removal (fixed factors) on percent survival in the selection experiment (parental plots). Each plot served as a statistical replicate.

Data from the reciprocal transplant experiment were used to investigate the effects of parental environment and offspring environment on leaf damage and fecundity in *Lotus* (Ellner et al. 2011), using a generalized linear mixed model. Insecticide application and *Medicago* removal in the parental environment and insecticide application and *Medicago* removal in the offspring environment were included as fixed factors. Parental plots, nested within the parental *Medicago* * parental insecticide interaction, and offspring plots, nested within the offspring *Medicago* * offspring insecticide interaction, were included as random factors in the model. The weights of seeds planted into the parental environment were included as a covariate to partially control for potential maternal effects resulting from differential resource allocation among maternal treatments. The model with the best-fit distribution was used in separate analyses on the proportion of leaves damaged in May and fecundity. All statistical analyses were performed using “proc glimmix” in SAS version 9.1 (SAS Institute, Cary, NC, USA, 2002–2003). Degrees of freedom were calculated using the Satterthwaite procedure.

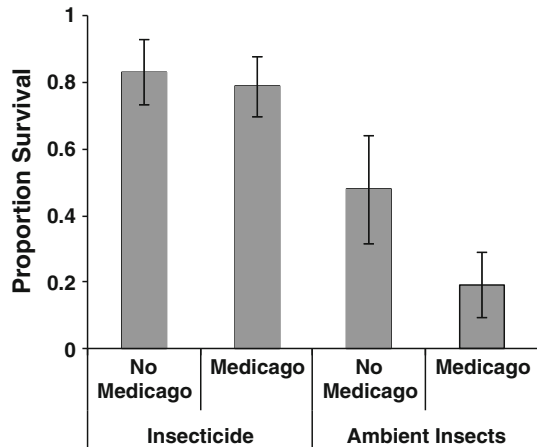
Significant effects of parental treatments indicate transgenerational effects, and significant effects of offspring treatments indicate more immediate ecological effects. Insecticide * *Medicago* removal interactions indicate indirect ecological (offspring treatments) or indirect transgenerational (parental treatments) effects. Interactions between parental and offspring treatments indicate that transgenerational effects altered the outcome of interactions in the offspring generation.

Results

Leaf damage, survival, and fecundity in the parental plots

The effect of insecticide and *Medicago* removal on leaf damage and fecundity (but not survival) in *Lotus* populations in the parental environment were reported in Lau and Strauss (2005). Insecticide decreased leaf damage by 63% and increased seed number by 329%. *Medicago* removal decreased leaf damage by 19% and increased seed number by

Fig. 1 Mean (\pm SE) proportion survival of *Lotus* from parental plots that manipulated densities of insect herbivores and invasive *Medicago*. Data from Lau and Strauss (2005) demonstrate similar effects on leaf damage and fecundity



128%, but only in the presence of insects, indicating an indirect effect (Lau and Strauss 2005). We found similar effects of insecticide and *Medicago* removal on survival. In the parental environment, insecticide increased *Lotus* survival ($F_{1,20} = 64.3$, $P < 0.001$; Fig. 1). *Medicago* removal also increased *Lotus* survival ($F_{1,20} = 7.72$, $P = 0.012$), but this effect was more pronounced with ambient insect abundance (insecticide**Medicago*: $F_{1,20} = 4.24$, $P = 0.053$), also suggesting indirect effects between insects and *Medicago* on *Lotus* survival (Fig. 1).

Lotus from parental environments with ambient densities of both insects and *Medicago* showed some of the same responses to insecticide and *Medicago* offspring environment treatments. As above, *Medicago* removal decreased leaf damage, but in this case, the effect of *Medicago* was independent of insects (Fig. 2A). No significant effect of insecticide on leaf damage was detected in offspring environments, even though insecticide significantly increased fecundity in offspring environments (Fig. 2B). This result suggests that the insecticide treatment was less effective in the offspring environment than in the parental environment, possibly because of the smaller plot size used in offspring treatments. A smaller plot size may have increased the likelihood of recolonization of the treatment plots from outside areas following insecticide applications.

Transgenerational effects alter leaf damage

Although the presence of *Medicago* in the offspring environment affected the amount of leaf damage experienced by *Lotus*, the strength of that effect was altered by transgenerational effects. The presence of *Medicago* in the offspring environment increased leaf damage on *Lotus*, but only for populations from parental environments with neither insects or *Medicago* or both insects and *Medicago* (Fig. 2A).

Because insecticide and *Medicago* removal treatments in the parental generation decreased *Hypera* herbivory on *Lotus*, one might expect to observe selection for more resistant *Lotus* in ambient insect parental treatments, especially when *Medicago* is also present and increases *Hypera* abundance. As a result, *Lotus* offspring from insect/*Medicago* present plots may have evolved increased resistance and experienced less *Hypera* herbivory than offspring from insecticide/*Medicago* absent plots. Likewise, if epigenetic effects contributed to transgenerational effects and *Lotus* induced defenses in offspring of

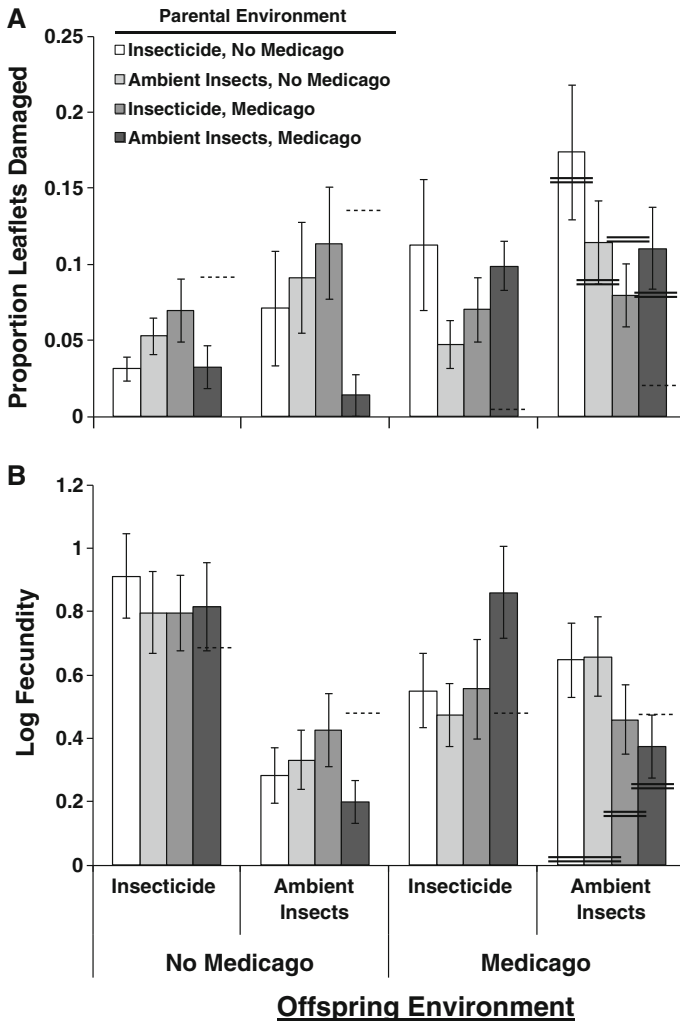


Fig. 2 The mean (\pm SE) proportion of *Lotus* leaflets damaged (**A**) and fecundity (**B**) from different parental environments, grown in different ecological (offspring) environments. *Double lines* represent the predicted additive ecological effects of insects and *Medicago* for *Lotus* from each parental environment. *Dotted lines* represent predicted additive transgenerational effects within each offspring environment. Differences between same-colored bars in different offspring environments represent ecological effects, while differences among bars within each offspring environment represent transgenerational effects

plants exposed to high amounts of herbivory, then one would expect *Lotus* offspring from insect/*Medicago* present plots to have higher induced defenses (and experience less *Hypera* herbivory) than *Lotus* offspring from insecticide/*Medicago* absent plots. Consistent with this prediction, we found that when grown in offspring plots with ambient insect densities, *Lotus* from insect/*Medicago* present parental plots experienced reduced herbivory compared to *Lotus* from insecticide/*Medicago* removal parental plots (Fig. 2A). Interestingly, the presence of both insects and *Medicago* in the parental generation were required to significantly reduce damage in offspring grown in environments in the absence of

Table 1 Results from 4-way generalized linear mixed models analyzing the effects of parental and offspring environments (\pm insects and \pm *Medicago* in each environment) on *Lotus* leaf damage and fecundity

Effect	May leaf damage			Fecundity		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Parental _{insects}	1,207	0.001	0.981	1,32	2.91	0.098
Parental _{Medicago}	1,201	3.73	0.055	1,31	0.020	0.880
Offspring _{insects}	1,6	1.48	0.272	1,14	2.02	0.177
Offspring _{Medicago}	1,6	3.80	0.102	1,14	2.020	0.177
Par _{ins} *Par _{Med}	1,204	0.950	0.332	1,32	0.230	0.635
Off _{ins} *Off _{Med}	1,6	0.120	0.745	1,15	0.110	0.746
Par _{ins} *Off _{ins}	1,207	0.590	0.444	1,214	1.21	0.272
Par _{Med} *Off _{Med}	1,200	0.020	0.880	1,214	1.60	0.208
Par _{ins} *Off _{Med}	1,207	1.20	0.2743	1,214	0.130	0.715
Par _{Med} *Off _{ins}	1,200	0.530	0.467	1,214	5.28	0.023
Par _{ins} *Par _{Med} *Off _{ins}	1,203	1.22	0.270	1,214	2.96	0.087
Par _{ins} *Par _{Med} *Off _{Med}	1,201	9.06	0.003	1,214	1.06	0.304
Par _{ins} *Off _{ins} *Off _{Med}	1,207	2.69	0.103	1,214	7.71	0.006
Par _{Med} *Off _{ins} *Off _{Med}	1,202	1.35	0.247	1,214	4.85	0.029
Par _{ins} *Par _{Med} *Off _{ins} *Off _{Med}	1,204	2.63	0.399	1,214	7.53	0.007
Seed weight	1,204	2.63	0.107	1,214	0.090	0.770

Italicized values represent marginally-significant effects ($P < 0.10$) and bold values indicate significant effects ($P < 0.05$)

Medicago, but the presence of either insects or *Medicago* in the parental generation was sufficient to reduce herbivory in offspring generations when offspring were grown in the presence of *Medicago* (Table 1, Fig. 2A).

Interactions between ecological and transgenerational indirect effects

There was an interaction between immediate indirect ecological effects (insecticide**Medicago* in offspring environments) and indirect transgenerational effects (insecticide**Medicago* in parental environments) on fecundity (Table 1, four-way interaction). This result implies that the fitness outcomes of interactions between *Lotus*, insect herbivores, and *Medicago* in the offspring generation depended on the presence of insect herbivores and *Medicago* in the parental generation (Fig. 2B). Ecological indirect effects on fecundity were strongest in *Lotus* from parental environments without *Medicago*, regardless of whether insects were present in the parental environment; fecundity in offspring environments with both insects and *Medicago* was different from that predicted by the additive ecological effects of each species (Fig. 2B, double lines). For *Lotus* from parental environments with *Medicago*, the strength of the ecological indirect effect was dependent on the presence of insects in the parental environment (Fig. 2B, double lines). This is indicative of an insecticide**Medicago* interaction in the parental environment (transgenerational indirect effect) that affects the strength of the insecticide**Medicago* interaction in the offspring environment (ecological indirect effect). Notably, these transgenerational effects on fecundity were not typically adaptive. Although offspring from insecticide/*Medicago* removal parental treatments tended to have higher fecundity than offspring from

other parental treatments when planted into insecticide/*Medicago* removal offspring treatments, this difference was not statistically significant. Moreover, for each of the remaining three offspring treatments, *Lotus* from the corresponding parental treatments did not have the highest fecundity.

Discussion

Our results demonstrate that both parental and offspring environments affect herbivory and fecundity of *Lotus*. The outcomes of species interactions between *Lotus* and competitors and herbivores are determined as much by the historical environment of the parents as they are by contemporary treatments, indicating the importance of transgenerational effects in ecological experiments. These transgenerational effects are driven either by maternal effects or rapid evolution following one generation of selection, or a combination of both effects. We manipulated the presence of multiple species, which allowed us to detect both direct and indirect interactions with other species; *Medicago* affected *Lotus* indirectly via effects on shared herbivores in both parental and offspring generations, demonstrating that indirect effects can have important transgenerational consequences that can affect the outcome of ecological experiments.

Effects of parental environment

Parental environment affected *Lotus* interactions in the offspring environments. In offspring environments with *Medicago* and ambient insect densities, *Lotus* from parental environments where both *Medicago* and insect herbivores had been removed experienced more leaf damage compared to *Lotus* from other parental environments (Fig. 2A). Alternately, in offspring environments with ambient insect densities, but without *Medicago*, removal of either *Medicago* or insects (or both) in the parental generation resulted in increased damage relative to *Lotus* from the parental treatments where both *Medicago* and insect herbivores were present. Thus, transgenerational effects of insecticide and *Medicago* removal resulted in increased herbivory in offspring generations.

These transgenerational effects also appeared to alter responses to experimental treatments in offspring generations. Consistent with prior work on this system, *Medicago* increased herbivory on *Lotus*, but only on *Lotus* from parental treatments with ambient insect and *Medicago* densities or insecticide/*Medicago* removal parental treatments. The presence of *Medicago* in the offspring environment did not increase herbivory on *Lotus* from parental treatments with ambient insect densities or ambient *Medicago* densities. Thus, in some cases, transgenerational effects of *Medicago* and insect herbivores reduced the ecological effects of *Medicago* on insect herbivory in offspring generations. These results are consistent with several recent studies that have demonstrated how transgenerational effects may alter the outcome of species interactions in subsequent generations (e.g. Agrawal et al. 1999; Meyer et al. 2006; Strauss et al. 2008; terHorst et al. 2010).

The strengths of transgenerational effects were not trivially small in this experiment, but rather their effects were on the same scale as those of more immediate ecological effects. For example, using the methods of Ellner et al. (2011), we found that the immediate ecological effect of insecticide decreased leaf damage by 33% and increased fecundity by 57% on average, while the transgenerational effect of insecticide increased leaf damage by 29% and decreased fecundity by 19%. Similarly, the immediate ecological effects of *Medicago* removal decreased leaf damage by 41% and decreased fecundity by 12%, but the

transgenerational effect of *Medicago* increased leaf damage by 18% and increased fecundity by 17%. With respect to both leaf damage and fecundity, transgenerational effects offset the immediate ecological effects of insecticide and *Medicago* removal. A failure to account for parental environment and transgenerational effects in this experiment would have resulted in significant over- or underestimation of true ecological effect sizes.

The transgenerational effects observed here may result from rapid evolution or maternal effects. In fact, both rapid evolution and epigenetic maternal effects are predicted to cause the herbivory responses observed here. Because *Medicago* increases the abundance of their shared herbivore, *Lotus* from insect/*Medicago* present environments are expected to receive increased herbivory. As a result selection on plant defenses may be stronger in those environments, resulting in the evolution of increased resistance (and decreased herbivory) for *Lotus* offspring from insect/*Medicago* present treatments. The increased herbivory in those treatments may also result in epigenetic maternal effects that cause increased defenses in *Lotus* offspring from insect/*Medicago* present treatments; such epigenetic effects also are predicted to cause decreased herbivory on offspring. Rapid evolution in this system is possible given that previous work found genetic variation in *Lotus* traits that mediate interactions with both *Medicago* and insect herbivores and that the presence of insects and *Medicago* altered selection on herbivore resistance and tolerance (Lau 2008). However, whether evolution or maternal effects, or both, are responsible for the transgenerational effects observed in this study is impossible to determine without further experiments. By including seed weight as a covariate, we attempted to account for differential provisioning of seeds among mothers that result in maternal effects. We found no significant effect of parental treatments on seed weight ($P > 0.13$), nor did we find qualitative differences when we removed seed weight as a covariate. However, epigenetic maternal effects would be unlikely to be detected by seed weight. Regardless of the underlying mechanism, our results suggest that transgenerational effects can alter ecological effects across one generation.

Indirect transgenerational effects

In addition to transgenerational effects imposed by direct species interactions, indirect transgenerational effects were important to contemporary *Lotus*-herbivore interactions. Consequently, the transgenerational response of *Lotus* to one biotic selection agent (*Medicago*) was often dependent on the presence of another biotic agent (insects) in the parental environment (Fig. 2). In our study, indirect transgenerational effects sometimes counteracted direct ecological or transgenerational effects of insect herbivores and may contribute to maladaptive transgenerational effects. In this study, fitness typically was not significantly higher when offspring environments matched parental environmental conditions, suggesting that the observed transgenerational effects were not typically adaptive. This lack of adaptation could be the result of variable environmental conditions and selection pressures from year to year. For example, in some years, *Medicago* only has a direct competitive effect on *Lotus* fitness, but in other years, *Medicago* affects *Lotus* fitness indirectly by increasing shared herbivore densities (Lau and Strauss 2005).

The diffuse evolution literature (sensu Janzen 1980) has explored the consequences of multispecies evolution and an emerging eco-evolutionary literature is beginning to explore the effect of evolution on ecological processes (Schoener 2011; Ellner et al. 2011). Our results highlight that understanding ecology in natural biological communities requires accounting for both direct and indirect effects (due to the multiple interactions that occur in

complex communities) and effects that span generations. Even relatively simple experimental manipulations can result in more complex dynamics than expected.

Acknowledgments We thank A. McCall, J. Wright, L. Yang, and T. Yang for field assistance. T. Bassett, K. Keller, R. Prunier, E. Schultheis, T. Suwa, D. Weese, and two anonymous reviewers provided valuable input on a previous version of the manuscript. This manuscript is based upon work supported by the National Science Foundation under grant # IBN-0206601 awarded to S.Y. Strauss and J.A.L. and DEB-0918963 awarded to J.A.L. This work was performed at the University of California Natural Reserve System's Donald and Sylvia McLaughlin Reserve. Data and the SAS code for the analyses presented in this manuscript can be found on Dryad (doi:10.5061/dryad.22672702). This is contribution #1588 from the Kellogg Biological Station.

References

- Abrams PA (1992) Predators that benefit prey and prey that harm predators: unusual effects of interacting foraging adaptation. *Am Nat* 140:573–600
- Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in plants and animals. *Nature* 401:60–63
- Bassar RD, Marshall MC, Lopez-Sepulcre A, Zondona E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc Natl Acad Sci USA* 107:3616–3621
- Bezemer TM, Thompson LJ, Jones TH (1998) *Poa annua* shows intergenerational divergences in response to elevated CO₂. *Glob Chang Biol* 4:687–691
- Conner JK (2003) Artificial selection: a powerful tool for ecologists. *Ecology* 84:1650–1660
- de Haan RL, Barnes DK (1998) Inheritance of pod type, stem color, and dwarf growth habit in *Medicago polymorpha*. *Crop Sci* 38:1558–1561
- Ellner SP, Geber MA, Hairston NG (2011) Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecol Lett* 14:603–614
- Huxman TE, Hamerlynck EP, Jordan DN, Salsman KJ, Smith SD (1998) The effects of parental CO₂ environment on seed quality and subsequent seedling performance in *Bromus rubens*. *Oecologia* 114:202–208
- Huxman TE, Charlet TN, Grant C, Smith SD (2001) The effects of parental CO₂ and offspring nutrient environment on initial growth and photosynthesis in an annual grass. *Int J Plant Sci* 162:617–623
- Irwin RE (2006) The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *Am Nat* 167:315–328
- Janzen DH (1980) When is it coevolution? *Evolution* 34:611–612
- Johnson MTJ, Stinchcombe JR (2007) An emerging synthesis between community ecology and evolutionary biology. *Trends Ecol Evol* 22:250–257
- Lau JA (2008) Beyond the ecological: biological invasions alter natural selection on a native plant species. *Ecology* 89:1023–1031
- Lau JA, Strauss SY (2005) Insect herbivores drive important indirect effects of exotic plants on native communities. *Ecology* 86:2990–2997
- Lau JA, Peiffer J, Reich PB, Tiffin P (2008) Transgenerational effects of global environmental change: long-term CO₂ and nitrogen treatments influence offspring growth response to elevated CO₂. *Oecologia* 158:141–150
- Meyer JR, Ellner SP, Hairston NG, Jones LE, Yoshida T (2006) Prey evolution on the time-scale of predator-prey dynamics revealed by allele-specific quantitative PCR. *Proc Natl Acad Sci USA* 103:10690–10695
- Miller TE (1994) Direct and indirect species interactions in an early old-field plant community. *Am Nat* 143:1007–1025
- Miller TE, terHorst C (forthcoming) Indirect effects in communities and ecosystems. In: Gibson D (ed) Oxford Bibliographies Online: Ecology. Oxford University Press, New York
- Miller TE, Travis J (1996) The evolutionary role of indirect effects in communities. *Ecology* 77:1329–1335
- Platenkamp GAJ, Shaw RG (1993) Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. *Evolution* 47:540–555
- Reale D, McAdam AG, Boutin S, Berteaux D (2003) Genetic and plastic responses of a northern mammal to climate change. *Proc R Soc Lond B* 270:591–596

- Roach DA, Wulff RD (1987) Maternal effects in plants. *Annu Rev Ecol Syst* 18:209–235
- Rossiter MC (1996) Incidence and consequences of inherited environmental effects. *Annu Rev Ecol Syst* 27:451–476
- Schoener TW (2011) The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* 331:426–429
- Slobodkin LB (1961) Growth and regulation of animal populations. Holt, Rinehart, and Winston, New York
- Stone L, Roberts A (1991) Conditions for a species to gain advantage from the presence of competitors. *Ecology* 72:1964–1972
- Strauss SY, Irwin RE (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annu Rev Ecol Evol Syst* 35:435–466
- Strauss SY, Lau JA, Schoener TW, Tiffin P (2008) Evolution in ecological field experiments: implications for effect size. *Ecol Lett* 11:199–207
- terHorst CP (2010) Evolution in response to direct and indirect ecological effects in pitcher plant inquiline communities. *Am Nat* 176:675–685
- terHorst CP (2011) Experimental evolution of protozoan traits in response to interspecific competition. *J Evol Biol* 24:36–46
- terHorst CP, Miller TE, Levitan DR (2010) Evolution of prey in ecological time reduces the effect size of predators in experimental microcosms. *Ecology* 91:629–636
- Thompson JN (1998) Rapid evolution as an ecological process. *Trends Ecol Evol* 13:329–332
- Walsh MR, Reznick DN (2010) Influence of the indirect effects of guppies on life-history evolution in *Rivulus hartii*. *Evolution* 64:1583–1593
- Wootton JT (1994) The nature and consequences of indirect effects in ecological communities. *Ann Rev Ecol Syst* 25:443–466
- Yoshida T, Jones LE, Ellner SP, Fussmann GF, Hairston NG (2003) Rapid evolution drives ecological dynamics in a predator–prey system. *Nature* 424:303–306
- Yoshida T, Ellner SP, Jones LE, Bohannan BJM, Lenski RE, Hairston NG (2007) Cryptic population dynamics: rapid evolution masks trophic interactions. *PLoS Biol* 5:1868–1879
- Youngson NA, Whitelaw E (2008) Transgenerational epigenetic effects. *Annu Rev Genomics Hum Genet* 9:233–257