

**IDEA AND
PERSPECTIVE**

Evolution in ecological field experiments: implications for effect size

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Abstract

Rapid evolution in response to strong selection, much of which is human-induced, has been indisputably documented. In this perspective, we suggest that adaptation may influence the effect size of treatments in ecological field experiments and alter our predictions of future dynamics in ecological systems. Field experiments often impose very strong and consistent selection over multiple generations. Focal populations may adapt to these treatments and, in the process, increase or decrease the magnitude of the treatment effect through time. We argue that how effect size changes through time will depend on the evolutionary history of the experimental population, the type of experimental manipulation, and the traits involved in adaptive responses. While no field study has conclusively demonstrated evolution in response to treatments with concomitant changes in ecological effect size, we present several examples that provide strong circumstantial evidence that such effects occur. We conclude with a consideration of the differences between plastic and genetic responses to treatments and discuss future research directions linking adaptation to ecological effect size.

Keywords

Contemporary evolution, ecological-evolutionary feedbacks, population dynamics, rapid evolution.

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Rapid adaptive evolution, usually studied in the context of human-caused selection on populations over decades, has now been well-documented (recently reviewed in May 2007 issue of *Functional Ecology*; Stockwell *et al.* 2003; Meyer *et al.* 2006; Strauss *et al.* 2006). Adaptation in experimental populations in response to ecological manipulations has also been shown (Snaydon & Davies 1982; Reznick & Ghilambor 2001; Reznick *et al.* 2004; Hairston *et al.* 2005). Experimental perturbations, even more than other human-induced changes, can cause large and consistent changes in the selective environment. In this perspective, we suggest that rapid adaptation to treatments, resulting from consistent differences in patterns of selection or opportunity for selection (expression of genetic variation in fitness) between treatments, may influence the effect size of treatments in ecological field experiments and our ability to predict future dynamics in ecological systems.

The need to consider feedbacks between ecological and evolutionary processes has been advocated repeatedly (e.g. Ford 1964; Antonovics 1976, 1992; Neuhauser *et al.* 2003); these feedbacks have been modelled in recent theoretical treatments (Abrams & Matsuda 1997; Loeuille

& Loreau 2004, 2005; De Mazancourt *et al.* 2005; Jiang *et al.* 2005) and explicitly addressed in a few experiments (e.g. Lankau & Strauss 2007; Yoshida *et al.* 2007). There are few data available, however, to assess how rapid adaptation to experimental treatments affects the often-measured ecological quantities of population size, biomass, or the rate of population growth (but see Hairston *et al.* 2005; Collins & Bell 2004). Similarly, while a number of studies have been specifically designed to assess the effect of prior history on ecological effect size (e.g. Pritchard & Schluter 2001; Collins & Bell 2004; Steiner *et al.* 2007), there are many more ecological studies that ignore how the evolutionary history of populations prior to the experiment may influence the response to treatments. Finally, while laboratory experiments in simplified microcosms show the potential for adaptation to alter predator–prey population dynamics (Hairston *et al.* 2005) or other species interactions (Bohannan & Lenski 2000), we are aware of no field experiment that has characterized how evolutionary responses over the course of an experiment influence interactions with other species or ecosystem processes.

Adaptation to both prior- and post-manipulation environments may affect responses to experimental treatments in both short-term and long-term field experiments [sometimes equated with ‘pulse’ and ‘press’ experiments, respectively (Bender *et al.* 1984)]. Short-term experiments, defined here as manipulations that are imposed within a generation, show the immediate response of a population to the treatment, and it has been argued, minimize impacts of indirect ecological effects (Schmitz 1998; Wootton 2002). Evolution may alter responses in short-term experiments if there is differential mortality among genotypes, and traits linked with survival also have effects on biomass, productivity, or other ecological responses. Multi-year/generation experiments, in contrast, provide an opportunity to examine effects of a manipulated variable when integrated across temporal variation in abiotic conditions and densities of interacting species. Adaptation in long-term experiments can occur both through differential mortality and through differential recruitment or reproduction. Responses to both short- and long-term experimental manipulations can also be affected by the evolutionary history of experimental populations, something that has been proposed as a potential explanation for the small effects sometimes observed in competitor- and herbivore-removal experiments (Harper 1969; Connell 1980; Schoener 1983; Schluter 2000; DeWalt *et al.* 2004; Steiner *et al.* 2007). Despite the potential for adaptation to alter effect size in ecological experiments, *to date no compelling empirical examples explicitly link the magnitude of ecological responses in field experiments to concomitant evolutionary change in traits.*

DEFINITION OF EFFECT SIZE

For the purposes of this paper, we use the term effect size to refer to the magnitude of a response to an experimental manipulation, and formally define effect size in Fig. 1 as the log of the ratio of the treatment mean to the control mean [that measure recommended by Hedges *et al.* (1999)]. Ideally, effect sizes would be measured in units of population density, population growth rate, population biomass, or productivity, to be most applicable to ecological dynamics, although other response variables can be measured as well (e.g. behaviour, herbivore damage, etc.). We are particularly interested in how population projections might change with ongoing adaptation to treatments.

EVOLUTIONARY HISTORY OF A POPULATION IS THE STARTING POINT FOR ADAPTATION

The evolutionary history of a population will determine the starting point from which populations may adapt to experimental treatments. Prior adaptation to one environ-

ment may limit or bias the range of genotypes available to respond to selection exerted by treatments, and can result in either underestimates or overestimates of long-term ecological responses. Two examples highlight the importance of evolutionary history. Steiner *et al.* (2007) found that clones of *Daphnia ambigua*, cultured from eggs in pond sediment collected prior to colonization by late-arriving *Daphnia* species, went extinct in laboratory competition experiments with these late-colonizing species. In contrast, *D. ambigua* clones that currently coexist with these competitors in the field were also able to coexist with them in the laboratory (Steiner *et al.* 2007). Thus, the effect size of *Daphnia* competitors on *D. ambigua* depends on the source/history of *D. ambigua* populations – whether the resurrected populations occupied the lake before or after colonization by competitors. Similarly, in two unpublished experiments conducted by Reznick and Endler, when guppies from an environment with a history of very low adult guppy predation were introduced to stream segments with high predation pressure on adult guppies, the experimental populations rapidly went extinct (Reznick, pers. comm.). The ecological effect of predation on completely naïve populations was much greater (extinction) than the effects of the same treatment when applied to populations of guppies with prior exposure to predators of juvenile guppies (multi-generation persistence of populations; Reznick *et al.* 2004). Conversely, if experimental treatments remove antagonists, short-term impacts of treatments on antagonist-adapted populations may be small (Lennartsson *et al.* 1997), but longer-term effects of antagonist removal may result in increased effect size, if organisms can adapt to capitalize on the antagonist-free environment, possibly by losing costly defences.

The role of history and its influence on effect size is not always clear-cut; for example, *a priori* we have no clear expectations for how a history with predators could affect the ability of individuals to adapt to mutualist species. We expect the effects of prior history to be most predictable when experimental manipulations are highly divergent from, or highly concordant with, prior history with respect to that manipulation (e.g. the effect of grazer removal on populations of previously ungrazed plants or on populations that have been historically grazed).

ADAPTATION IN EXPERIMENTAL MANIPULATIONS

In the examples above, naïve populations went extinct before they could adapt to new competitors or predators. In the following paragraphs, we describe examples in which ongoing adaptation to experimental treatments occurs, or seems likely, and in which adaptation may affect estimates of treatment effects.

Very long-term experiments: grasses at Rothamsted

In the Park Grass experiments at Rothamsted, adaptation has occurred in response to a diversity of experimental soil mineral additions over periods ranging from 6 to 112 years. Liming treatments, begun in 1920, increased soil pH and typically reduced the yield of the perennial grass *Anthoxanthum odoratum* (Snaydon 1970, Snaydon & Davies 1972, 1982). Adaptation by *A. odoratum* to these treatments had occurred within 50 years such that genotypes from the liming treatments were less affected by liming than genotypes from control (no lime) plots (Snaydon 1970; Snaydon & Davies 1972, 1982). Moreover, when 16 plots that had not been limed (but had received a variety of other soil treatments for decades) were split into adjacent, newly limed and unlimed (control) treatments, populations of *A. odoratum* adapted to minimize the detrimental effects of new liming treatments within 6 years (generations) (Snaydon & Davies 1982; Silvertown *et al.* 2005). Similarly, in longer-running experiments, *A. odoratum* genotypes collected from plots with 112 years of P fertilization exhibited significantly greater biomass (by up to 20%) when fertilized with P relative to genotypes from no P-addition treatments. Thus, *A. odoratum* populations adapted to capitalize on P-availability, which resulted in an increase in the effect size of P addition on biomass through time. In all of these grass experiments, maternal effects were reduced or removed by growing tillers of all genotypes in a common environment prior to assessing effects of treatments. The results from the Park Grass experiments are also interesting because they show that adaptation occurred even in the face of gene flow across treatments, indicating that proximity of experimental

environments is not a reason to assume that adaptation will not occur.

Elements of these Rothamsted experiments were generally conducted over very long time scales, and the question remains whether adaptation to treatment typically occurs within more standard long-term field experiments of 6–10 years and few generations. Unlike in many natural systems (Grant & Grant 2002), the direction and magnitude of selection in experiments is often large and consistent,

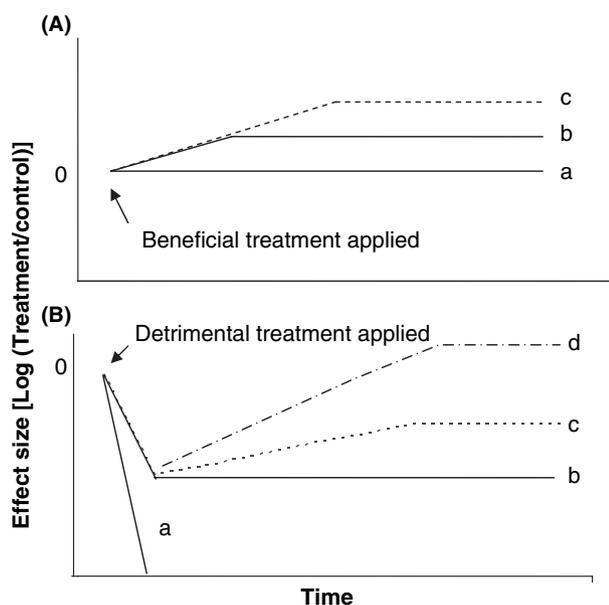


Figure 1 Panels A and B represent some of the many possible ecological and evolutionary responses to experimental treatments, and are perhaps simplistic as they ignore constraints on population size owing to other density-dependent factors and costs of adaptation. Regardless, the panels illustrate how evolution can alter the ecological effect size of a treatment over time; effect sizes also change with adaptation when the more complicated processes described above are included in predictions (Tiffin 2000; Yoshida *et al.* 2007). Panel A represents possible changes in effect size on population variables (e.g. population size, growth rate, productivity, biomass) in response to a treatment that is beneficial (release from antagonist or beneficial manipulation like fertilization). Curves are drawn as linear, but could take on a number of functions. All treatments start at the same initial conditions. (a) Insufficient adaptive plasticity or lack of genetic variation leads to no response to beneficial manipulation, perhaps owing to prior history (e.g. release from competitor results in no response if prior adaptation has reduced detrimental effects of competitor); (b) plastic immediate response to manipulation leads to rapid treatment effect (short-term and long-term effects similar). (c) Benefits from manipulation increase over time through long-term plastic responses or adaptation, or both (e.g. Snaydon & Davies 1972, 1982) (long-term experiments provide different estimates of effect size from short-term experiments). Panel B depicts changes in response to a detrimental manipulation: (a) insufficient adaptive plasticity or lack of genetic variation in a naïve population leads to extinction; (b) reduction in effect size owing to adaptive plasticity but no subsequent adaptation – short-term and final effect size remain the same, (c, d) short-term reduction in population size with subsequent plasticity plus adaptation by survivors to antagonist; in this case, the short-term effect of the manipulation is greater than the long-term effect moderated by plasticity and/or adaptation; (c) the antagonist treatment still reduces final population size relative to populations with no antagonist; (d) adaptation to antagonist results in an eventual positive, rather than negative, effect. Population sizes after adaptive evolutionary change could end up smaller than initial population sizes if costs of adaptation outweigh benefits of escape from antagonists (e.g. Yoshida *et al.* 2007; not depicted). In both panels, plateaued responses in the absence of evolutionary change may occur because of limits in adaptive plasticity, because indirect effects or other density-dependent ecological factors become limiting. Adaptive responses may plateau due to the previous ecological factors, plus limited genetic variation, fitness costs, or changes in the selective environment caused by manipulation.

although its strength may vary with the type of treatment. Removal of ambient levels of an interactor may be more variable in strength than experimental treatments using regular additions or subtractions of fixed amounts (nutrient or predator additions per capita of prey). For example, during a 6-year interval the mean annual ambient leaf damage levels from herbivores on *Raphanus sativus* in the field differed significantly among years and ranged from 9.6 to 17.9% (Strauss, unpubl. data); thus, herbivore removal treatments in the field likely had greater selective effects in some years than in others. Despite variation in the strength of selection from some treatments, numerous studies over short time scales have demonstrated differences in patterns of selection on traits as a result of experimental manipulations of the presence of: herbivores (e.g. Mauricio 2000, Pilon 2000), pollinators (Galen & Cuba 2001), predators (Losos *et al.* 2004), competitors (Lau *et al.* 2007), combinations of interactors (Gomez 2003; Rudgers & Strauss 2004; Irwin 2006; Lau 2006) or intensity of abiotic stress (Stanton *et al.* 2000; Steinger *et al.* 2003). In many of these studies, treatments also affected the predicted evolutionary response by influencing the opportunity for selection [i.e. the expression of genetic variation in fitness (e.g. Stanton *et al.* 2000)]. Below, we give some tantalizing examples in which evolutionary responses to changes in selection may occur during ecological experiments substantially shorter than those at Rothamsted, and in which ecological effect size may change concomitantly.

Evolutionary changes in response to an elevated CO₂ environment

Understanding how plants respond to elevated CO₂ (eCO₂) is important because these responses may alter interspecific interactions and because increases in plant biomass can act as biological sinks for carbon and can buffer against the impacts of rising CO₂ (Pepper *et al.* 2005). The growth of *Lupinus perennis* plants propagated from seeds collected from populations grown in ambient (aCO₂) or eCO₂ environments for 5 years prior in a free-air CO₂ enrichment experiment suggests that evolution may contribute to decreasing effects that eCO₂ has on biomass accumulation through time. Specifically, although genotypes collected from both aCO₂ and eCO₂ treatments were larger when reared in eCO₂ compared with aCO₂ environments, genotypes collected from aCO₂ showed a much larger biomass increase in response to eCO₂ than did genotypes collected from eCO₂ (55% vs. 29% greater biomass for aCO₂ and eCO₂ populations, respectively; Lau and Tiffin, unpubl. data). These differences are likely genetic: similar effects were detected when seed weight was included in statistical models in an attempt to minimize non-genetic maternal effects. Because genotypes collected from long-

term eCO₂ treatments show a weaker biomass response to eCO₂, predictions based on short-term responses to eCO₂ could overestimate the ability of natural plant communities to act as carbon sinks. Differing magnitudes of response to CO₂ over time are generally attributed to physiological acclimatization, change in a limiting resource, plastic changes, or indirect effects – the potential role of evolutionary change is seldom considered.

Evolutionary changes may affect population dynamics

In a few studies, ignoring evolution has impeded a full understanding of ecological dynamics. Evolution affects oscillations in population densities of rotifer predators and algal prey in laboratory experimental microcosms (Yoshida *et al.* 2003, 2004). When prey populations were stocked with a single algal genotype (minimal opportunities for evolution), algae and rotifers exhibited short-term predator–prey phase oscillations as predicted by theory; in contrast, in microcosms started with multiple algal genotypes, algae and rotifers exhibited much longer population cycles, and oscillations were often out of phase (Yoshida *et al.* 2003, 2004); differences in cycling were attributed to evolution in algal prey populations. Similar experiments in a bacteria/phage system show that predator–prey cycles between bacteria and phage ‘predators’ in microcosms disappear completely when evolution is allowed to occur. Lack of cycling occurs because resistant bacterial strains are selectively favoured over susceptible strains in the presence of phage, and these do not cycle with phage (Yoshida *et al.* 2007). Thus, overall bacterial population dynamics changed from cycling to constant densities as a result of evolution in response to phage attack.

In field experiments, similar results obtain. Reznick *et al.* have shown that guppies in high predation environments exhibit different life histories (size at maturity, investment in reproduction, senescence, and age at first reproduction) from guppies in low predation environments (reviewed in Reznick & Ghalambor 2005). Laboratory rearing experiments demonstrate that guppies from replicated high and low-predation environments exhibit genetically based differences in life-history traits. When guppies are experimentally released from predation, reversion to low-predation life-history occurs within 4–7 years or *c.* 7–13 generations (Reznick *et al.* 2004). Life-history adaptations to predation can significantly alter population growth rates of guppies in natural environments (Bronikowski *et al.* 2002); preliminary data on long-term effects of predation on guppy populations appear to integrate effects of predation rate, guppy adaptation to predators and costs of those adaptations, and release from intraspecific competition under high predation (Bronikowski *et al.* 2002; Reznick & Bryant 2007).

Using predator introductions on islands, Schoener, Losos and Spiller combined experimental field manipulation of predation pressure with measurements of selection on prey, and concomitant estimates of the ecological effect size of predation through time (Schoener *et al.* 2002; Losos *et al.* 2004, 2006). Selection on *Anolis sagrei* limb length changes with predator addition treatments and limb length is both plastic and heritable in this species (Calsbeek & Smith 2007). Unfortunately, the two sets of experiments from which these data were collected, although perfectly set up to quantify the effect of adaptive evolution in limb traits on ecological effect sizes of predators, were prematurely terminated by hurricanes before the interplay between adaptation and population dynamics could be fully assessed.

Evolution in a focal species may affect interactions with other community members

Evolution in one species over the course of an experiment might alter interactions with other community members. As part of a large experiment designed to understand interactive effects of CO₂ enrichment, nitrogen addition, and biodiversity, Reich *et al.* (2001) planted *Lespedeza capitata* from a common seed source into monocultures or plots with 12 species. In 2004, seven generations after initial planting, Lau *et al.* (2007) found that plants growing in monoculture plots were significantly more pubescent (hairy) than plants growing in high diversity treatments ($F_{1,27} = 15.47$, $P = 0.0005$). A 23% difference in pubescence was also observed in plants reared from seeds collected from these plants and grown in a common greenhouse environment ($F_{1,11} = 5.36$, $P = 0.04$), suggesting that the difference in pubescence is genetic rather than plastic, although maternal effects cannot be ruled out. The increased pubescence was negatively correlated with damage from generalist chewing herbivores in the field (Lau *et al.* 2007), and differences in pubescence and other plant traits explained 28% of the observed increase in generalist herbivory in high diversity plots compared with monocultures (plants in high-diversity environments experienced more generalist herbivore damage than plants in monocultures). Thus, the evolutionary change in pubescence may have increased the effect size of the diversity treatment on herbivore damage.

Adaptive plasticity vs. adaptive evolutionary change and effect size

The most immediate organismal responses to a large change in the direction of selection are likely plastic changes in behaviour, growth, or other induced responses that can occur rapidly and persist for long periods (Mathis *et al.* 1996; Underwood 2000; Proulx & Magnan 2004; Duquette *et al.* 2005; Ferrari *et al.* 2005; Degenhardt & Lincoln 2006). If

plastic responses to an ecological manipulation are rapid, we expect that there will be little difference between the short-term and long-term effects of that manipulation in the absence of any evolutionary change. However, even plastic responses can require time. For example, cross-generational maternal effects (Sultan 1995; Rossiter 1996; Lundgren & Sultan 2005; Rogilds *et al.* 2005; Bashey 2006) appear after reproduction and thus may also contribute to differences in the magnitude of responses to an experimental treatment in short-term and long-term experiments, even in the absence of evolution. Note, too, that populations can harbour genetic variation in plastic responses (Underwood 2000; Proulx & Magnan 2004; Duquette *et al.* 2005); thus, plasticity may evolve within experimental treatments. Another important difference between purely plastic and evolutionary responses is that plastic responses are likely to be more rapidly reversible (e.g., Zufali & Rausher 2004), as they do not involve any changes in the genetic composition of a population: changes in the genetic composition of a population require time for differential mortality, recruitment, or reproduction.

Future directions and experimental approaches

Rapid evolution by a diversity of organisms over decades has occurred in cases of herbicide and pesticide application, human over-harvesting, trophy hunting, the presence of introduced species, defences against natural enemies, and specialization of predators and parasites (e.g. Carroll *et al.* 1997; Thompson 1998; Conover & Munch 2002; Coltman *et al.* 2003; Conover *et al.* 2005; Phillips & Shine 2006). In many cases, the nature of adaptation in response to these pressures is predictable: the acquisition of herbicide and pesticide resistance, early and smaller age at maturity when larger individuals are selectively harvested, smaller 'racks' on sexually mature trophy animals, etc. Our intent is to illustrate the potential for similar rapid evolution to alter the magnitude of treatment effects in ecological experiments when these experiments consistently alter the nature of selection over multiple generations. Furthermore, in both long-term and short-term experiments, the evolutionary history of the study population prior to the experimental manipulation may strongly influence at least the initial magnitude of the treatment effect, and likely also the trajectory of subsequent evolution. Adaptation may also affect interactions with other species. How adaptation affects ecological effect size has received very little attention, as typically, evolutionarily oriented studies measure changes in traits and individual fitness, but not population responses as a whole. Similarly, ecologically oriented studies seldom consider evolutionary responses.

There are several important processes that could temper the effects of adaptation to treatment on population size,

growth rate or productivity. First, evolution will only occur if populations harbour genetic variation for traits associated with fitness in the novel environment. For example, while many plant species have colonized toxic mine tailings and show rapid adaptation to toxic soils, other plant species that are abundant in nearby communities fail to colonize tailings. The main difference between species able to colonize tailings and species that fail to colonize appears to be the presence of genetic variation for heavy metal tolerance in nearby populations (Bradshaw 1991). Second, ecological experiments could have unconsidered impacts on the population genetic structure of the experimental population. Treatments may alter breeding phenology or utilize small, isolated populations; both of these attributes may cause increased inbreeding or reduced effective population sizes, and these impacts may unrealistically promote or suppress adaptive responses. Third, and importantly, even if adaptation to treatment occurs, increased individual fitness may not translate into population-level increases in N , growth rate or population biomass. Ecologically, compensatory mortality and other density-dependent processes may negate changes in populations, even if individuals have adapted to treatments and have increased individual fitness. Evolutionarily, changes in traits may themselves alter population dynamics. For example, evolution of costly defences in response to increased predator or pathogen pressure may reduce absolute fitness (while increasing relative fitness) thereby causing reductions in host population sizes (Tiffin 2000; Yoshida *et al.* 2007). Conflicts between levels of selection also can counterintuitively diminish population fitness, even as individual fitness increases [e.g. conflicts between individual selection and group selection, conflicts between natural and sexual selection, or 'Darwinian suicide' (Fiegna & Velicer 2003; Webb 2003)]. The number of studies addressing whether evolutionary changes modulate population-level responses to ecological manipulations is too few to know when these effects will occur and how strong these effects will be.

Ideally, one would like to be able to predict how adaptive evolution affects ecological effect size. One possibility is that adaptation will be most important when the ecological perturbation has a large effect (but not so large that it is outside of what populations have experienced in the past). Larger experimental perturbations could magnify fitness differences between genotypes and thereby increase evolutionary potential. If perturbations are too large, however, populations may be driven extinct before adaptation occurs [as in Steiner *et al.*'s (2007) experiments with *Daphnia*, and Reznick and Endler's (unpubl. data) guppy experiments]. In the Park Grass experiment described earlier, the level of genetic divergence between treatments after 6 years of experimental manipulation was correlated with the magnitude of ecological effect, indicating that treatments with the

largest ecological effects will at least sometimes result in large evolutionary responses (Snaydon & Davies 1982). Surprisingly, there appear to be few data available to evaluate whether larger ecological perturbations result in more rapid evolution of ecologically important traits.

Another expectation is that populations will respond to antagonistic or stress-causing manipulations differently than beneficial manipulations. When experimental manipulations dramatically decrease fitness, we predict a large initial ecological effect and a decline in population size, assuming population sizes are related directly to fecundity. If a population harbours genetic variation for a trait that would lessen these fitness impacts, that trait will be expected to evolve rapidly, thereby reducing the magnitude of the manipulation's initial effect on population sizes (bottom panel, Fig. 1). So, for an introduced competitor, we expect character displacement to decrease impacts on fitness, and for a novel predator, we expect the evolution of anti-predator behaviours or morphology that would reduce the ecological impact of predation. In contrast, when an experimental treatment is beneficial (fertilization, release from antagonist), we predict an increase in the magnitude of the treatment effects as selection favours genotypes that can best take advantage of the beneficial manipulation (top panel, Fig. 1; results of Rothamsted experiment on P-addition described above). Again, for population size to change with adaptation to treatment, we assume that adaptation results in increases in absolute, as well as relative fitness, and that the effects of adaptation are not counterbalanced by population regulation from other density-dependent factors, or by costs of adaptations. Changes in effect size will ultimately reflect the interplay of trait changes, accompanying costs of adaptation, covariance between the trait and the ecological response variable, and altered ecological interaction strengths resulting from adaptation.

Testing for an evolutionary contribution to ecological responses

The paucity of studies attempting to quantify the effect that adaptation has on ecological effect sizes does not appear to be due to experimental intractability – experimental investigation of the interplay between adaptation and ecological effect sizes should not be particularly onerous, at least in some systems. Either vertical or horizontal approaches can be employed to assess evolutionary contributions to ecological responses. A vertical approach would require comparing the ecological effect size on genotypes present at the beginning of an experiment to genotypes present at the end of an experiment. This approach would, of course, be possible only with organisms in which propagules can be preserved (e.g. plant seeds, *Daphnia*, bacteria, etc.). In this

case, careful controls for vigour loss and maternal effects would entail at least one generation in a common environment prior to assessing response to treatments in early and late populations. A horizontal approach, in contrast, would involve assaying a representative sample from populations randomly assigned to different treatments at the end of (or midway through) an experiment. Again, it will be important to minimize any maternal effects and account for the contribution of plastic responses. Therefore, for either vertical or horizontal approaches, the contribution adaptation has made to effect sizes could be estimated by (1) sampling genotypes (either at the beginning and end in the case of vertical approach, or from control and treatment environments in the horizontal approach); (2) growing or raising these genotypes in a common environment to minimize maternal effects and plastic responses; and (3) assaying genotypes (or their progeny) by putting representatives from each treatment back into all experimental environments. If genotypes collected from controls show different responses to manipulations than those collected from other treatments, or if genotypes from the beginning of the experiment show different population responses from later genotypes, then evolution within treatments may contribute to the total ecological response. The magnitude of these effects could also be assessed at this point. Finally, to include the impact of evolutionary history in these responses, one could include multiple source populations in the experiment that differ in their history with the manipulated variable.

CONCLUSIONS

Increased awareness of the importance of the selective environment prior to imposing an ecological treatment and the potential for adaptation to occur during an ecological experiment could improve our understanding of population and community responses to experimental perturbations; these considerations can also be important in correctly identifying the mechanistic basis underlying the effects of experimental manipulations. Only multi-year experiments can estimate the long-term effects of ecological perturbations, including the role that evolution plays in response to ecological manipulations. Multi-generation experiments will also incorporate commonly found cross-generational plastic responses (i.e. maternal, paternal or grand-parental effects). Data from studies examining evolutionary responses to ecological manipulations and the impact those evolutionary changes have on the ecological response are needed to determine when evolutionary responses are most likely to occur and how evolutionary responses alter interpretation of results or long-term predictions. Future work in long-term experiments could also address basic questions in evolutionary ecology that have received little attention, including:

- (1) How often do adaptive responses to treatments alter effect sizes in ecological experiments?
- (2) What is the relationship between the magnitude of ecological and evolutionary responses?
- (3) How do evolutionary changes affect reversibility (whether a system can return to its pre-manipulation state) and responses to future changes in selective pressures?

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