Intensified agriculture favors evolved resistance to biological control

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Increased regulation of chemical pesticides and rapid evolution of pesticide resistance have increased calls for sustainable pest management. Biological control offers sustainable pest suppression, partly because evolution of resistance to predators and parasitoids is prevented by several factors (e.g., spatial or temporal refuges from attacks, reciprocal evolution by control agents, and contrasting selection pressures from other enemy species). However, evolution of resistance may become more probable as agricultural intensification reduces the availability of refuges and diversity of enemy species, or if control agents have genetic barriers to evolution. Here, we use 21 years of field data from 196 sites across New Zealand to show that parasitism of a key pasture pest (Listronotus bonariensis, Argentine stem weevil) by an introduced parasitoid (Microctonus hyperodae) was initially nationally successful, but then declined by 44% (leading to pasture damage of c. NZD 1600 m p.a.). This decline was not attributable to parasitoid numbers released, elevation or local climatic variables at sample locations. Rather, in all locations the decline began 7 years (14 host generations) following parasitoid introduction, despite releases being staggered across locations in different years. Finally, we demonstrate experimentally that declining parasitism rates occurred in ryegrass Lolium perenne, which is grown nationwide in high-intensity pastures, but not in adjacent plots of a less-common pasture grass (Lolium multiflorum), indicating that resistance to parasitism is host-plant dependent. We conclude that low plant and enemy biodiversity in intensive large-scale agriculture may facilitate the evolution of host resistance by pests and threaten the long-term viability of biological control.

Attack rates | GAMM | invasive species | meta-analysis | natural enemy

Introduction

Global human population growth demands increased food production (1). This increasing need has led to increases in agricultural monocultures, which exacerbate yield losses to pest species (2, 3). Moreover, rapid evolution of pest resistance to chemical control (4), combined with the negative impacts of pesticides on human health and the environment, have increased calls for sustainable and acceptable pest management methods (5-7). Biological control of pests by native and introduced natural enemies is an ecosystem service worth billions of dollars annually (8), and it has been heralded as a powerful solution due to its low cost and long-term effectiveness, if initial control is achieved (9). Although pest evolution of resistance to microbial control agents has been documented (10), there are few if any examples of evolved resistance to introduced parasitoids or predators (11, 12), even though heritable variation in resistance to parasitoids exists and could be selected upon if the benefits outweigh any costs of resistance (13).

Several hypotheses can explain this absence of resistance (11). First, co-evolutionary arms races (natural enemies evolving counter-adaptations to the pest) may prevent host resistance from occurring (14, 15). Second, spatial and temporal refuges from attacks may reduce the overall selection pressure on the host, or allow source-sink evolutionary dynamics whereby vulnerable genotypes are maintained by immigration from refuges (16). In addition, combinations of different enemy species may exert separate selective pressure, and thereby prevent the pest from evolving resistance to any single enemy across its entire range (17).

However, these mechanisms that prevent resistance to biological control could in theory be undermined in large-scale homogeneous agricultural systems, which may have few refuges to sustain susceptible strains of the pest, low variability in attack rates, and low biodiversity of enemy species (9). Moreover, co-evolutionary arms races may favor one participant if mutation or recombination rates, or even available genetic diversity, differ significantly between enemy and pest. This could occur due to differences in population bottle-necks (e.g., if few enemy individuals are introduced) or in sexual versus asexual reproduction (18).

We therefore hypothesize that the conditions associated with agricultural intensification and expansion could favor the evolution of host resistance to biological control agents. Here, we use 21 years of data from a well-studied interaction between an exotic pest species [Listronotus bonariensis (Kuschel) (Coleoptera: Curculionidae)], Argentine stem weevil and its introduced parasitoid [Microctonus hyperodae Loan (Hymenoptera: Braconidae)] in exotic pasture in New Zealand to test whether parasitism shows changes congruent with this hypothesis. The pest was self-introduced, first discovered in 1927 and by the 1980s was causing NZD $74-251 million of damage per annum (19). A

Significance

The need for agricultural production to meet the food demands of a growing human population will require sustainable and acceptable pest management, such as biological control, across 11% (1.5 billion ha) of the globe’s land surface. However, the long-term viability of this ecosystem service can be threatened by the expansion and simplification of agricultural systems, which may facilitate the evolution of resistance by pests to their control agents. This study uses a national dataset to present evidence for the acquisition of resistance by a ryegrass weevil pest to its parasitoid wasp over the last 21 years. This resistance was not associated with differences in environmental conditions, but rather is specific to the most commonly-grown pasture grass species.

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parasitoid species from its native range was introduced in 1991 and provided successful control with peak parasitism rates of 80-90% in the early years (20–22). However, there has been emerging evidence of recent declines in attack rates (23, 24). An obvious hypothesis is that these declines could be driven by abiotic (e.g., climate) or biotic (e.g., parasitoid-related) variables altering the host-parasitoid interaction (25). Alternatively, several conditions present in large-scale intensified pasture ecosystems suggest that evolved resistance to the parasitoid may have been possible during the c. 50 generations L. bonariensis has undergone since the first releases of its parasitoid (26).

Firstly, the parasitoid is parthenogenetic and suffered a severe population bottleneck during introduction [only 132 individuals comprising clonal lines from 7 geographically separate populations were released into the country (21)]. Thus, we hypothesize that these factors would place it at an evolutionary disadvantage against the host, which undergoes sexual recombination during each of two generations a year (27), and theoretical work has shown that constraints to parasitoid evolution could rapidly lead to the evolution of resistance by hosts (28). Secondly, the spatial and temporal variability in attack rates that typically prevents the evolution of resistance (11, 26) may be reduced in crops with low species diversity, structural simplicity and a large, connected spatial continuum such as New Zealand’s improved pastures. These occupy c. 10.6 ha of New Zealand [c. 40% of the total land area (29)]. In particular, c. 29% of improved pasture is intensively managed with low species diversity compromised of predominantly perennial ryegrass L. perenne, often with a single species of white clover, Trifolium repens. Such large-scale production systems of low cultivated diversity are common (e.g., monoculture cash crops, plantation forestry), and when the crop is structurally simple, as is often the case, spatial refuges from attack may be scarce.

Finally, control agent resistance to natural enemies is rare because diverse enemy assemblages in pests’ indigenous ranges typically inflict varied selection pressure, such that no enemy species singularly exerts enough pressure (i.e., mortality) to drive the evolution of resistance (11). Again, this barrier to resistance may be reduced if enemy diversity is lower in high-intensity agricultural systems. For example, in New Zealand pastures,
grazing intensity is associated with a decline in the diversity and abundance of spider predators, which have approximately half the diversity of the fauna from similar sites in England (30), and invertebrate predators generally have low impact on L. bonar-iensis populations in New Zealand (31, 32). Insectivorous birds also have low abundance in the absence of native vegetation on New Zealand farms. Further, this vegetation (along with the proportion of ‘unimproved’ low-intensity pasture) has declined with intensified farm management over the past century (33).

Hence, we hypothesize that this lack of alternative predation pressure, coupled with initially high parasitism rates, would also have imposed a strong selection pressure on the weevil population, further accelerating the evolution of resistance.

We begin by reporting on a significant decline in L. bonar-iensis parasitism by M. hyperodae in the last 21 years, and we examine whether this pattern is more consistent with variation in abiotic or biotic conditions or with the hypothesis of acquisition of resistance by the weevil to the parasitoid. We then explore whether parasitism rates are more similar in sites that co-occur spatially, or whether the release date of the parasitoid (i.e. time available for the acquisition of resistance) better explains present-day similarities in attack rates across sites. Subsequently, we use a field experiment to test the hypothesis that declining attack rates are specific to the predominant grass species used in intensified pastures, as indicated earlier in greenhouse studies (34).

Results

Long-term declines in field parasitism rate

We found that overwintering parasitism rates declined significantly (rho = 0.68, P < 0.001) with time elapsed since the first parasitoid release. The best-fitting model was a cubic smoothing spline (λ = 1.34, Df = 2), with breakpoint analysis highlighting the significant (P < 0.001) presence of two breakpoints (seven years and 12 years since first the release of the parasitoid; Fig. 1).

The best-fitting model with lower AIC belonged to the GAMM family (Table 1) and a comparison with this model is presented in Table S1, indicating that both a non-linear relationship with time and the inclusion of region-level random effects improved model fit. Within the GAMM family, the best-fitting model (GAMM1; AIC = 863.6) indicated that the years elapsed since first release had a significantly negative, but non-linear effect (P < 0.001) on overwintering parasitism rates. The GAMM1 results also indicated that the first year of parasitoid release had a significantly positive, but non-linear effect on parasitism rates (P < 0.001). There was no significant interaction effect between release date and time since release (Table S1), indicating that parasitism rates at a given location began to decline seven years post release, irrespective of the release date at that particular site. None of the remaining predictors included in the model (year of sampling, total number of parasitoid individuals released, several measures of local climate, elevation, or a spatial autocorrelation parameter) showed any significant relationship with parasitism rates (Table 1).

Determination of parasitism rates: ordination and pattern

The NMDS ordination indicated that trends in the parasitism rates clustered together according to the major regional release dates (Fig. 2). A gradient was observed whereby there was a cluster of points associated with the 1991 parasitoid releases and another cluster that comprised the five regions (see Fig. S1 for a map with the region codes given in Fig. 2) in which the parasitoid was released later (i.e. 1993, 1995, 1996 or 1998). This cluster of later-release-date sites also contained one region where there was a low number of parasitoids released (< 1000 individuals; KA) and three where the parasitoid was not released and had instead arrived later through natural dispersal (MB, SC and WN; Fig. 2).

Overall, even sites that were spatially highly separated and climatically very different (with different amounts of pasture in the region) grouped together (e.g. sub regions such as MC and BP, respectively on the dry east and wet west coasts of the South Island grouped with RI, TO, WO and AK of the central and northern North Island, because all received parasitoid releases in 1991 or later via natural dispersal). This demonstrates that attack rates declined through time at different sites based on the local date of parasitoid release rather than their spatial proximity. Finally there was another cluster generated by regions where the parasitoid was released in 1995 or 1996 (i.e. BP and HB) but where few or no L. bonar-iensis were found in the recent sampling campaigns (Fig. 2).

Field experiment: influence of pasture type on parasitism rate

Fig. 3 shows rates of parasitism in our experimental plots, measured at fortnightly intervals during the period 2014-2016. Notably, this frequency of sampling accommodated the large fluctuations in parasitism rates that occur with interacting population dynamic processes of both the weevils and the parasitoid (35). Regardless, there were significant differences in the rates of parasitism on the two grass types (RSS = 1222.3, P < 0.001) and significant similarity in the pattern of parasitism rates (Cross-correlation = 1, P = 0.001).

It could be possible that differences in host abundance across grass species were the cause of parasitism differences, rather than the consequence of differences in attack rates. However, previous work showed that the searching efficiency of the parasitoid was very high even at very low weevil ground densities (36), which suggests that density-dependence is unlikely to explain the patterns observed here. Nevertheless, to test specifically for dependence on host density we used historical data from Goldson, Proffitt and Baird (26), and tested for a correlation between host abundance and parasitism rate. This revealed no significant relationship (p = -0.1, P = 0.5), which suggests that attack rates were unlikely to have been host density dependent.

Discussion

It has been argued that biological control provides sustainable long-term pest suppression, because the diversity of selection pressures, refuges from attack, and the co-evolutionary arms races that are present in most ecosystems prevent the evolution of resistance to their natural enemies (11, 17). Indeed, the time and expense associated with pre-release testing of control agents is predicated on an assumption that rapid evolution of resistance is unlikely. Yet, here we have demonstrated a significant decline in control rates of an economically-important pest by its introduced parasitoid control agent. Importantly, the measured decline was not associated with local abiotic conditions, nor did it originate in one location and subsequently spread to other nearby locations. Rather, the declines began simultaneously across the country, seven years (approximately 14 host generations) after release of the parasitoid at each given location (22), and reached a plateau at present-day attack rates after 12 years. A seminal experimental study of host-parasitoid evolution (37) found similar marked and rapid declines in attack rates (by 40-68% in 8-20 generations, depending on the specific experimental treatment), and these declines were consistent across replicates, as they were across locations in our study. Given our findings of national-scale uniformity in time to resistance, and the lack of spatial clustering in resistance patterns, it is unlikely that resistance occurred via the spread of a novel mutation. Rather, it likely involved a selective sweep on resistant genotypes that existed at lower frequencies in the background population, however, this hypothesis requires testing with genomic techniques.

The mechanism of resistance is not yet clear, however, there is sufficient evidence to posit several hypotheses. First, there may...
have been the evolution of some form of escape behavior, as
behavioral change can rapidly generate new phenotypes (38). For
example, a study of field crickets (Teleogryllus oceanicus) on the
Hawaiian islands showed that genetically-based resistance in this
species had occurred twice involving separate genetic changes
on different islands in the same archipelago (39). The crickets
stopped stridulating (after about 24 generations) because such ac-
tivity attracted the parasitic fly (Ormia ochracea) and this species
exerted negative selection pressure. The potential for escape
behavior in Argentine stem weevil has been suggested by previous
work which showed that, in the presence of the parasitoid, the
weevils tended to knock off the foliage towards the soil in upright
potted plants (40). This leads to the hypothesis that plant physical
structure may affect the ability of the weevil to employ its escape
behavior. If correct, this would suggest that resistance could be
related to the 3-dimensional structure of the dominant plant
species (in this case, L. perenne). However, this hypothesis was not
supported by a more recent study by Goldson and Tomasetto (34),
which showed that parasitism rates did not differ significantly
between vertically- versus horizontally-positioned grass tillers in
laboratory cages. Thus, in spite of an apparent lack of impact
of plant structure, plant species did indeed significantly affect
parasitism rates. Goldson and Tomasetto (34) showed that in
the presence of L. perenne, parasitism rates were significantly
lower (c. 46%) than in the presence of the far less common
short-rotation pasture species L. multiflorum (c. 75%), whereas
empty control cages showed 35% parasitism. These laboratory
results accord with the field experimental results presented here,
wherein parasitism rates were lower in weevils on L. perenne than
in L. multiflorum (Fig. 3). If these differences are not caused
by plant structure, they may be caused by differences in plant
chemistry such as volatiles, which can be important in attracting
natural enemies of herbivores (41). Although L. multiflorum
alone has been shown experimentally not to be attractive to our
parasitoid species M. hyperodae (42), plant feeding by the weevil
may nevertheless stimulate the release of herbivore-induced plant
volatiles that elicit a parasitoid or weevil response. This requires
further investigation.

An alternative hypothesis for the mechanism of resistance is
encapsulation of the parasitoid egg by the host immune system
(43). However, encapsulation is unlikely to have generated the
observed differences in parasitism rates across host-plant treat-
ments (in both the field and laboratory), and in fact no evidence
of parasitoid encapsulation has been observed despite thousands
of weevil dissections by numerous workers (35).

Whatever the mechanisms of resistance are, our finding that
attack rates on weevils remained higher (though with the same
seasonal dynamics) in experimental plots of an uncommon pas-
ture species (L. multiflorum) may provide a possible opportunity
to off-set the impacts of resistance of the weevil to its parasitoid.
Specifically, given the evidence that the mode of resistance is
host-plant dependent, there may be opportunities for the intro-
duction of pasture-species diversity, which may allow attack rates
to approach their previous levels. At the same time this study’s
finding also serves as a warning that low crop diversity (such as
the single species of grass dominating New Zealand pastures) may
facilitate adaptation by pests to their enemies.

To our knowledge, there are no clear cases of evolved resis-
tance to introduced biological control parasitoids (11, 17, 44),
and the only other possible example may have been caused
by the introduction of new host strains (45). Irrespective, the
absence of evidence may not be evidence of absence. Insuffi-
cient post-release monitoring of biological control introductions
means that long-term efficacy of control remains unclear (46).
Although not necessarily deliberately selected for in biologi-
cal control, parthenogenetic parasitoids are common within the
Hymenoptera and are therefore sometimes used in biological
control (47). Moreover, the typically low natural enemy diver-
sity in intensified agriculture is likely to increase the selection
pressure imposed by control agents (48). Both factors increase
the likelihood of evolved resistance. Thus, we hypothesized
that any sustained success of biological control introductions will
be lowest in situations where the agent is parthenogenetic, crop
biodiversity is low, the crop is grown over a large spatial extent,
the pest and control agent are specific to a single crop type, and
there has been considerable time since parasitoid release. Even in
the absence of long-term monitoring, these hypotheses could be
explored, for example using a meta-analysis of biological control
parasitism rates worldwide. Moreover, our results suggest that
pre-release assessments should consider the available variation in
pest susceptibility (on which selection could operate), the genetic
diversity of agents being released, and the diversity of existing
enemies (i.e., sources of alternative selection pressure).

Biological control has the potential to be a sustainable
method of long-term pest suppression. However, its efficac
depends on a suite of mechanisms that prevent the appearance
of resistance to parasitoids and predators. These mechanisms
may break down in intensive agro-ecosystems with low biodiver-
sity (17). Although resistance to insecticides is explicitly managed
against, the same is not presently true for biological control using
predators and parasitoids, and we hope that our findings will
stimulate discussion on this topic. Agro-ecosystem biodiversity
offers a variety of benefits for biological control (49), such as
resources for natural enemies and greater pest suppression via
enemy diversity (50, 51). In addition, crop and enemy biodiversity
may be crucial for the maintenance of co-evolutionary regimes
that prevent the resistance of pests to their natural enemies, and
maintain the multi-billion dollar ecosystem service of biological
control (5).

Materials and Methods

Data collection and extraction

We assembled published data on the percent parasitism of L. bonariensis
by M. hyperodae collected from 18 New Zealand biological ‘sub-regions’ (52)
from 1994 to 2015 (data will be made available to readers upon request). The parasitism rates used were measured during overwintering diapause
time periods, a total of 336 published and unpublished records of M. hyperodae parasitism of L. bonariensis from all of the sampled regions of New Zealand were used (51). Firstly, we extracted the parasitism rates and the collection dates (i.e. years) from graphs (e.g. scatter plots or histograms) using DATATHIEF III software (http://datathief.org/).

Where these data were not available in the publication, we obtained them directly from the corresponding authors of the studies (4 contacted, 4 replied). We supplemented these published data with unpublished data obtained by dissecting frozen archived weevil samples as part of ongoing national parasitism surveys over the last 21 years. In total, these data were obtained primarily from weevils collected in Lolium spp. pastures across 196 sites. This amounted to examining by dissection a total of c. 11,000 individual
weevils. The dissections for parasitoid eggs and larvae in all data sources followed the protocol used in published studies elsewhere (55).

Temporal and spatial analysis of field parasitism rate

We used this 21-year weevil parasitism dataset to examine whether attack rates changed significantly over time, whether any changes were
time-linear, and whether they could be explained by parasitoid-related factors or local environmental conditions rather than adaptation by the host. To
achieve this final model, we sequentially determined specific aspects of a model chosen through a process described below.
First, to characterize any temporal trend in the overwintering parasitism rates, we investigated different fitting lines to the data, and this process selected a cubic smoothing spline with a polynomial fit. Because there were duplicated points in the response variable, i.e., different studies measuring parasitism rates in the same location and at the same date, we also applied a generalized cross-validation method in order to reduce any potential bias.

We based the final model on a logistic model implemented through a quasi-binomial generalized linear model using R software (R 3.3.1; http://www.r-project.org). Among the models (Table 6) and for each of the additional package ’gam’. This was extended by adding a random effect (v) in the linear predictor to account for possible error due to the nested structure (arising from the different regions in New Zealand) in a generalized linear mixed-effects model (GLMM). Visual inspection of the data suggested non-linear changes in parasitism through time, so we allowed for the possibility of a non-linear influence of time by using a generalized additive model (GAM) using the function ’gam’. This latter model family was extended to include random effects in the predictor terms via a generalized additive mixed model (GAMM) using the function ’gam’. We ran a second version of the GAMM data depicting similarity and/or dissimilarity within or between the assemblages when datasets cannot be presumed to consist of an assumed probability of correlations of standardized environmental variables to their unit variance via the ’rankindex’ function. We then selected the NMDS model with the lowest stress (i.e., the best model fit) using the ’metaMDS’ function.

To classify the New Zealand sub regions into different clusters, we specifically used a dendrogram generated by hierarchical clustering with the distance between cluster centroids (i.e., single linkage method) as the preferred method to correctly reproduce the actual estimated distance within the field study plots. Subsequently, we compared the spatial autocorrelation originating from the potential movement of parasitoids in a spatial auto-covariate term (SAC). In our case, the SAC term accounted for spatial autocorrelation to affect the parameter estimates and error sub regions, we used the ’envelf’ function with 10,000 permutations and then fitted significant vectors (with P ≤ 0.05) which were overlaid onto the ordination.

Field experiment: influence of pasture type on parasitism rate

Previsous evidence suggests that the predominant plant species used in pastures may have played a part in the observed reduction in parasitism. In a field experiment, we observed the field data of L. bonariensis by M. hyperodae was significantly higher in plots comprising a less common grass species (L. multiflorum) than in plots comprising the commonly-grown L. perenne. Further laboratory experiments confirmed that parasitism rates did differ according to the host-plant present (34).

Here we extended the field experiment of Goldson, Tomsetto and Popay (35), with an additional year of sampling specifically to explore potential temporal variability in the mean parasitism rates. Detailed methods can be found in Goldson, Tomsetto and Popay (35), but the experiment is briefly summarized here. The experimental plots were sown in 2013 on the AgResearch Lincoln Research Farm (43.631788, 172.449383) and comprised L. multiflorum (cv. Lush AR73 fugal endophyte) and the L. perenne (cv. Lincoln) which were both sown on September 2013 and established well. The plots were set-stocked with lambs throughout the winters. Nitrogen was applied as urea on four occasions (in spring 2013, summer/autumn 2014, in early December 2014 and early January 2015).

We tested for statistical significance between the mean parasitism rates in the L. multiflorum vs. L. perenne plots. Because the normality assumption for a traditional one-way analysis of variance (ANOVA) was not met, we used a non-parametric complete random permutation test in cycles = 10,000 for a one-way ANOVA via the additional package ’lmPerm’. In addition to dealing with violation of the normality assumption, this provides a flexible and intuitive methodology for statistical analysis and implements the methods for permutation tests described by Kabacoff (62).

Moreover, the multiple time-series data obtained were analyzed using a cross-correlation analysis built in the additional package ’xtseries’. This analysis measured the extent of similarity of two series (Cross-correlation = 1) as a function of the lag of one relative to the other. This allowed estimation of the extent to which temporal trends in parasitism rates differed in L. multiflorum vs. L. perenne.

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