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 y 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. 160 million New Zealand dollars per annum). 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 y (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 was significantly less than 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

Global human population growth demands more food production (1). This expanding demand 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, has 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 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, coevolutionary arms races (natural enemies evolving counteradaptations 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 pressures, 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, coevolutionary 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 bottlenecks (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 y 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 74–251 million New Zealand dollars of damage per annum (19). A parasitoid species from its native range was introduced in 1991 and provided successful control, with peak parasitism rates of 80 to 90% in the early years (20–22). However, there has been emerging evidence of recent declines in

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 y. This resistance was not associated with differences in environmental conditions but rather is specific to the most commonly grown pasture grass species.
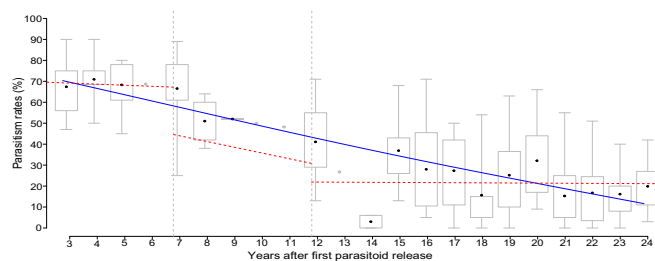
Fig. 1. Decline in overwintering *L. bonariensis* parasitism by *M. hyperodae* between 1994 and 2015. Best fit using GAM (i.e., cubic smoothing spline with equivalent df = 2; solid blue line) and three OLS-CUSUM models fitted to the data (dashed red lines) that account for estimated breakpoints (7 and 12 y since first release of the parasitoid; dashed gray lines) are shown. Gray points represent individual plots in years where data were missing, which were estimated via cubic smoothing spline with a polynomial fit; individual mean values of parasitism rates (%) are shown by black points, the 25th to 75th percentile is shown by gray boxes, and the range of values is shown by the lines outside the gray boxes.

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).

First, the parasitoid is parthenogenetic and suffered a severe population bottleneck during introduction [only 132 individuals comprising clonal lines from seven 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). Second, the spatial and temporal variability in attack rates that typically prevents the evolution of resistance (11, 28) 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 million 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 comprised of predominantly perennial ryegrass *Lolium 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. bonariensis* 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. bonariensis* parasitism by *M. hyperodae* in the last 21 y, and 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 significantly higher in 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 [$\lambda = 1.34$, degrees of freedom (df) = 2], with breakpoint analysis highlighting the significant ($P < 0.001$) presence of two breakpoints (7 and 12 y since first release of the parasitoid; Fig. 1). The best-fitting model with lower Akaike information criterion (AIC) belonged to the generalized additive mixed model (GAMM) family (Table 1), and a comparison with this model is presented in Table S1 indicating that both a nonlinear relationship with time and the inclusion of region-level random effects improved model fit. Within the GAMM family, the best-fitting model (GAMM$_1$; AIC = 863.6) indicated that the years elapsed since first release had a significantly negative but nonlinear effect ($P < 0.001$) on overwintering parasitism rates. The GAMM$_1$ results also indicated that the first year of parasitoid release had a significantly positive but nonlinear 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 7 y postrelease, 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).

Table 1. Results of the best-fitting overwinter parasitism rates with explanatory variables recorded at each site via the quasibinomial generalized additive mixed model

Variables	GAMM$_1$
Intercept[†]	−1.1*
Years after first parasitoid release[‡]	−0.1***
Year of sampling[†]	−0.1
First year of parasitoid released[‡]	0.1***
No. of parasitoids released[†]	0.1
Elevation,[†] m	0.1
Mean annual precipitation,[†] mm	−0.1
Growing degree days[†]	−0.1
Spatial autocorrelation term[†]	−0.1
AIC[§]	863.6

The full results of a comparison (using AIC and df = 309) with other regression model families are presented in Table S1. Asterisks refer to the statistical significance of the explanatory variable (***$P < 0.001$, *$P < 0.05$).
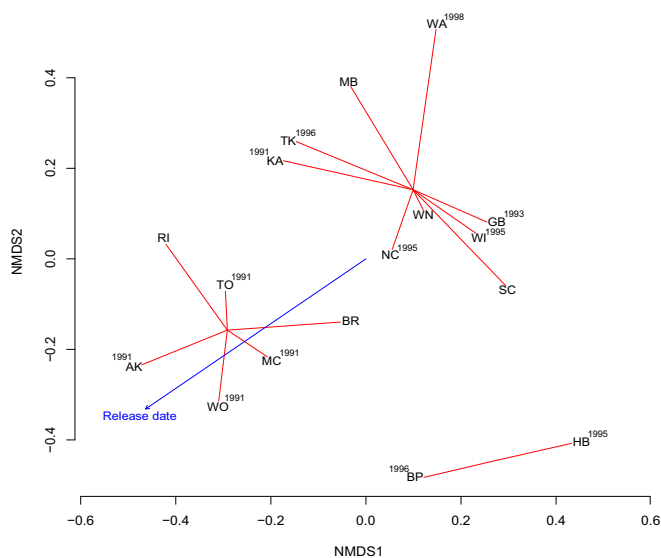[†]Nonsignificant variables.
[‡]Significant variables.
[§]The lowest AIC.

Fig. 2. NMDS ordination of New Zealand subregions [sensu Crosby et al. (52)] according to the temporal trend of overwintering *L. bonariensis* parasitism by *M. hyperodae* between 1994 and 2015. For graphical reasons, the resulting clusters were added to the ordination of the plots and connected to the group centroid using the function *ordispider*. Where dates are shown, these represent the years of the first releases of *M. hyperodae*. No dates are shown where no release of *M. hyperodae* was made.

Determination of Parasitism Rates: Ordination and Pattern. The nonmetric multidimensional scaling 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 versus 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 (<1,000 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., subregions such as MC and BR, 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 parasitism 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. bonariensis* 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 to 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 across the two grass types (residual sum of squares = 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 ($\rho = -0.1$, $P = 0.5$), 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 coevolutionary 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 prerelease testing of control agents is predicated on an assumption that rapid evolution of resistance is unlikely. However, 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, 7 y (~14 host generations) after release of the parasitoid at each given location (22), and reached a plateau at present-day attack rates after 12 y. A seminal experimental study of host–parasitoid evolution [e.g., fly host–wasp parasite, (37)] found similar marked and rapid declines in attack rates (by 40 to 68% in 8 to 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, although 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).
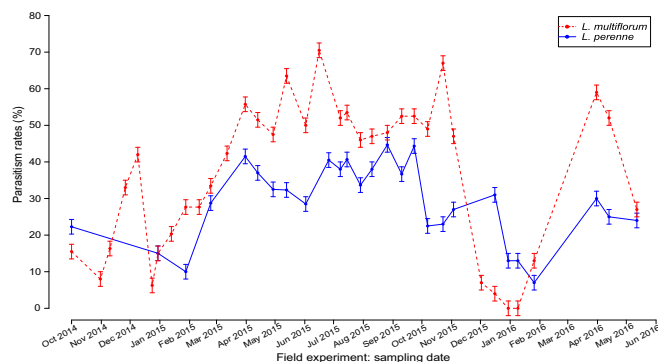
Fig. 3. *M. hyperodae* parasitism (%) of *L. bonariensis* collected between October 8, 2014 and May 19, 2016 from plots containing *L. multiflorum* [cultivar (cv.) Lush AR37 fungal endophyte; dashed red line] and *L. perenne* (cv. Samson endophyte-free; solid blue line) at the AgResearch Lincoln Research Farm. Values are the mean ± SEM of >14 individual weevils.

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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 activity attracted the parasitic fly (*Ormia ochracea*), and this species exerted negative selection pressure. The potential for escape behavior in the Argentine stem weevil has been suggested by previous work that showed that, in the presence of the parasitoid, the weevils tended to move off the foliage toward the soil in upright potted plants (40). This leads to the hypothesis that plant physical structure may affect the ability of the weevil to use its escape behavior. If correct, this would suggest that resistance could be related to the three-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, despite 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 significantly lower in weevils on *L. perenne* than on *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 treatments (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 (although with the same seasonal dynamics) in experimental plots of an uncommon pasture species (*L. multiflorum*) may provide a possible opportunity to offset 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 introduction 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 resistance 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. Insufficient postrelease monitoring of biological control introductions means that long-term efficacy of control remains unclear (46). Although not necessarily deliberately selected for in biological control, parthenogenetic parasitoids are common within the Hymenoptera and are therefore sometimes used in biological control (47). Moreover, the typically low natural enemy diversity 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 hypothesize 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 prerelease assessments should consider the available variation in pest susceptibility (on which selection could operate), genetic diversity of agents being released, and 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 efficacy depends on a suite of mechanisms that prevent the appearance of resistance to parasitoids and predators. These mechanisms may break down in intensive agroecosystems with low biodiversity (11, 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. Agroecosystem 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 coevolutionary regimes that prevent the resistance of pests to their natural enemies and maintain the multibillion-dollar ecosystem service of biological control (8).

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 "subregions" (52) from 1994 to 2015 (data will be made available to readers upon request). The parasitism rates used were measured during overwintering diapause when levels were found to have stabilized [e.g., (20, 53)]. Parasitism rates during the New Zealand summer months are known to fluctuate greatly due to the interplay between episodes of weevil emergence and varying parasitoid attack rates driven by its own patterns of emergence [e.g., (20, 23)]. Conversely, diapausing overwintering parasitism rates are stable, as there is neither any weevil eclosion nor adult parasitoid activity (53). We were therefore able to use these diapause-collected parasitism data as a conservative proxy measure of overall parasitoid activity and impact. There are different times of onset of both weevil and parasitoid postdiapause activity in the North and cooler South Island areas, due to differences in the rates of heat accumulation above a temperature development threshold of c. 10 °C (54). Therefore, parasitism data were collected between May and August inclusively in the North Island and May and September inclusively in the South Island. Data were available for all of the 21 y of this study except for four years (1997, 2001, 2002, and 2004).

Based on the above 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 (*Supporting Information*, *Further Reading*). First, we extracted the parasitism rates and the collection dates (i.e., years) from graphs (e.g., scatterplots or histograms) using DataThief III software (datathief.org/). Where these data were not available in the publication, we obtained them directly from the corresponding authors of the studies (four contacted, four 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 y. 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-y weevil parasitism dataset to examine whether attack rates changed significantly over time, whether any changes were 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 to reduce any potential bias.

We based the final model on a logistic model implemented through a quasibinomial generalized linear model using R software (R 3.3.1; cran. r-project.org; accessed April 10, 2016) and the function *lme* in the additional package gamm4. This was extended by adding a random effect (γ) 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 nonlinear changes in parasitism through time, so we allowed for the possibility of a nonlinear 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 using the function *gamm*. We ran a second specification for all of these families of models (i.e., GLMMs, GAMs, and GAMMs) by introducing an interaction term between the years elapsed since first release and the first year of release. To compare the suitability of the models, we used the Akaike information criterion.

Based on the parameterization discussed above, we were able to generate an appropriate model with which to test for the effects of abiotic and biotic variables on parasitism rates. We fitted binomial models with quasilikelihood to account for the overdispersion and to explain the proportion of overwintering parasitism (y, converted to the linear predictor η via a logit link function). We modeled this as a function of (*i*) biotic variables: the first year of parasitoid release, total number of parasitoid individuals released, year of sampling, and years elapsed since first release in that subregion; and (*ii*) abiotic variables: elevation extracted from the digital elevation model, mean annual precipitation and growing degree days above 10 °C at the location from which the data were collected. The climate data were obtained using National Institute of Water and Atmospheric Research climate maps.

During these analyses of parasitism rates over time, it was also possible to demonstrate the adequacy of an autoregressive model of order 1, AR (1), to account for temporal autocorrelation (56). Such adequacy was confirmed by an inspection of the standardized residuals, their autocorrelation function, and the *P* values of the Ljung–Box statistic (57). To capture this temporal autocorrelation, we therefore included the AR (1) component in all of the subsequent models.

Our sampling design (Fig. S1) meant that there was also the potential for spatial autocorrelation to affect the parameter estimates and error probabilities such that they could distort the variance–covariance matrix. To test for this, we used a Moran's *I* test using the spdep package and found low but significant autocorrelation in the parasitism rates (Moran's *I* = 6.95, *P* < 0.001). To account for this, we adjusted our models by including a spatial autocovariate (SAC) term. In our case, the SAC term accounted for spatial autocorrelation originating from the potential movement of *M. hyperodae* and its host *L. bonariensis* between sampling sites. We computed the SAC term for each sampling location using a neighborhood boundary of 300 km, weighted by inverse distances among neighboring observations and visually assessing the degree to which our models accounted for unexplained spatial variation by plotting a semivariogram of the normalized residuals.

Because our model indicated nonlinear changes in parasitism, we tested whether parasitism rates changed abruptly over the duration of this study and attempted to identify the year(s) in which any abrupt changes occurred. To do this, we fitted linear models by ordinary least squares (OLS) regression to the data and an OLS-based CUSUM [cumulative sums of standard OLS residuals process (58)] to investigate possible structural changes in the models (i.e., breakpoints). This analysis was conducted using the strucchange package. A formal test for the presence of breakpoints was conducted by adopting the methodology developed by Bai and Perron (59) through a minimization of the residual sum of squares with a Bayesian information criterion.

Determination of Parasitism Rates: Ordination and Pattern.

If there were strong local effects on parasitism rates (e.g., due to environmental conditions or local population genetics), we would expect to see parasitism rates grouping together according to their spatial proximity. To explore this possibility, we measured whether the temporal trend in parasitism rates (expressed as a site × site distance matrix obtained from a site × year matrix in which cells represented parasitism rates) was most similar when sites were spatially clustered (i.e., when they occurred in the same subregion of New Zealand) or when they had received released parasitoids in the same year.

To determine this, we used nonmetric multidimensional scaling (NMDS) implemented in the additional vegan package. NMDS is useful when datasets cannot be presumed to consist of an assumed probability distribution, as in this case. Moreover, the NMDS technique graphically depicts similarity and/or dissimilarity within or between the assemblages of clusters. We selected the most suitable distance matrix (i.e., Jaccard dissimilarity index) that best separates the subregions using the rank orders of correlations of standardized environmental variables to their unit variance via the *rankin- dex* 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 subregions 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 dendrogram (i.e., cophenetic distance). To optimize the classification for a given number of clusters (i.e., number of classes), we tested the *K*-means clustering using 100 iterations and random starts, optimized with Hellinger transformation as the standardization (60). The resulting classes were added "manually" to the ordination graph.

To test which of the abiotic or biotic variables were related to the clusters of sites identified by their ordination according to New Zealand subregions, we used the *envfit* function with 10,000 permutations and then fitted significant vectors (with $P \leq 0.05$), which were overlaid onto the ordination.

Field Experiment: Influence of Pasture Type on Parasitism Rate.

Previous evidence suggests that the predominant plant species used in pastures may have played a part in the observed reduction in parasitism. In a preliminary study (35), we observed in the field that parasitism 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 differed according to the host plant present (34). These findings contrasted with unpublished data obtained from the same experimental laboratory conditions in the 1990s, which suggested that at that time parasitism rates did not differ in the presence of the two grass types. Combined, these results implied that the loss of parasitoid efficacy in the intervening years may be specific to the *L. perenne* plants but less so in the *L. multiflorum* plants. From this it follows that, if plant species is an important mediator of evolved resistance, the weevil would not have had the opportunity to effectively adapt to *L. multiflorum* (34).

Here we extended the field experiment of Goldson, Tomasetto, 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, Tomasetto, 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, 172.464938) and comprised *L. multiflorum* (cv. Lush AR37 fungal endophyte) and *L. perenne* (cv. Samson endophyte-free) plots. Previous experiments had found no effect of *L. perenne* cultivars and endophytes on field parasitism levels of Argentine stem weevils (35). The treatments comprised two sets of 35 × 50-m plots that were sown in 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, early December 2014, and early January 2015).

The *L. multiflorum* and *L. perenne* plots were sampled fortnightly from October 8, 2014 until May 19, 2016 using a modified leaf-blower vacuum (Echo; 21cc) to suck pasture litter into a removable net recessed in the inlet pipe (61). Collections were made by dragging the machine across each plot for 15 min. The weevils were then removed from the litter and dissected to determine parasitism rates. A minimum sample size of weevils for dissection was set at 14 individuals (35).

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 nonparametric complete random permutation tests (*n* cycles = 10,000) for a one-way ANOVA via the additional package lmPem. In addition to dealing with violation of the normality assumption, this approach 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 tseries. 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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