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22. We assumed an inverse rank correlation between colony growth and the fraction of workers lost to diploid males. We assumed a zero-truncated Poisson distribution of matings per female (*15*), and that for any simulation that there were *n* sex-determining alleles that were each at the equilibrium frequency of 1/*n* in the population (*17*). The average sample size in our cohorts was 178 colonies with, on average, 60 slow-growing and 78 fast-growing colonies. We randomly assembled a population of 178 colonies, produced by queens that mated a variable number of times with males whose alleles at the sex-determining locus were randomly derived from the population; queens were necessarily heterozygous. For each colony, we calculated the proportion of diploid males produced and ranked the decrement in colony growth. After ranking the colonies by growth rate, we took that slowest growing and fastest growing subset (60 and 78 colonies, respectively) and calculated relatedness based on the number of matings by each queen. We calculated the difference in relatedness for each of 1000 replicates for populations that had from 3 to 20 sex-determining alleles in the population. We estimated the 95% confidence intervals of this difference from the simulated distribution of differences. Because all colonies are assumed to survive, even those that lose 50% of their worker force to diploid males (and they are more likely to have the highest relatedness), the differences that we obtain in simulations are likely to be larger than differences that will occur in nature. Colony mortality will censor the extreme data. The upper 95% confidence interval does not overlap the average relatedness difference.
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30. We thank X. Ren, C. Heymann, J. Vilinski, J. Yencharis, K. Miller, and the Bureau of Land Management in Grand Junction, CO, for assistance and J. Strassmann, D. Queller, I. Billick, and T. McGlynn for valuable comments. We acknowledge the support of NSF grants DEB-9509312 and IBN-9507470 (B.J.C. and D.C.W.), NSF grant BSR-9108034 (D.C.W.), the University of Houston Coastal Center, and a University of Houston Limited Grant-in-Aid of Research.

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Landscape Structure and Biological Control in Agroecosystems

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Biological pest control has primarily relied on local improvements in populations of natural enemies, but landscape structure may also be important. This is shown here with experiments at different spatial scales using the rape pollen beetle (*Meligethes aeneus*), an important pest on oilseed rape (*Brassica napus*). The presence of old field margin strips along rape fields was associated with increased mortality of pollen beetles resulting from parasitism and adjacent, large, old fallow habitats had an even greater effect. In structurally complex landscapes, parasitism was higher and crop damage was lower than in simple landscapes with a high percentage of agricultural use.

Understanding of species interactions is essential for implementation of biological control of insect pests. Also the number, shape, and spatial arrangement of habitat patches affect phytophagous and entomophagous insects differentially, and the trophic-level hypothesis of island biogeography predicts that relative importance of natural enemies increases with habitat area and decreases with habitat isolation (*1*).

We present effects of landscape structure on parasitism of the rape pollen beetle (*Meligethes aeneus*) and bud damage caused by this pest in agricultural landscapes of Northern Germany. Rape pollen beetles were attacked by three univoltine larval parasitoids, *Tersilochus heterocerus*, *Phradis interstitialis*, and *Phradis morionellus* (Hymenoptera, Ichneumonidae), of which the last is rare. *Phradis interstitialis* mostly attacks host larvae in the second instar, and *T. heterocerus* attacks host larvae particularly in the third instar. Both of these parasitoid species kill their host after the host larvae drop to the ground before pupation in the soil (*2*). A few species of insect predators are known but rare, such as ladybeetles, lacewings, and malachiid beetles (*2*, *3*). We varied the scale of our agroecological analyses, combining the more classical view focusing on local improvements in populations of natural enemies (*4*) with analyses at large spatial scales—the landscape scale (*5*, *6*). We met the inherent problem of landscape comparisons (many variables change simultaneously) by analyzing experimentally exposed crop plots in addition to crop fields.

Our studies focused not only on small-scale effects of field margin strips (*7*), but also on medium-scale effects of large fallows adjacent to rape fields (*8*). We mapped plant species richness, vegetation cover, and plant height on an area of 30 m^2 in each field margin strip and each fallow. On the landscape scale, we compared the effects of structural complexity of 15 landscapes characterized by a gradient from extremely simple and structurally poor landscapes, to complex and structurally rich ones with up to 50% uncultivated habitats (*9*).

Landscape complexity varies due to the variable intensity of agricultural practices, and this correlates with characteristics such as the nutrient richness of soils. We addressed this problem of confounding variables by analyzing potted rape plants in addition to rape crop fields (*10*). These experimentally exposed rape plots were established in the same local environment, had the same soil type, nutrient and water availability, and were planted with the same crop variety.

The type of field margin did not affect parasitism at the edge of rape crop fields. In contrast, parasitism in the center of the fields was enhanced by old field margins (Fig. 1A). Parasitism was about 50% at the edge of all fields. Toward the center of the fields it dropped significantly to 20% when only 1-year-old strips or no strips surrounded the fields, but when the field margins were 6 years old the parasitism rate was as high as at the field edge. In stepwise multiple regression analysis, neither host density, plant species richness, vegetation cover, nor plant height in the field margins could be used as predictors of rates of parasitism. Because parasitoid populations hibernating in the soil are known to be negatively affected by agricultural practices like ploughing (*11*), and beetles hibernate mainly in forest areas (*12*), only these old and undisturbed strips enabled populations of parasitoids to build up over years and to enhance parasitoid dispersal into the fields (*13*).

Beetle larvae in rape adjacent to large old fallows had rates of parasitism even greater

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than those near old field margins. Parasitism of larvae on summer rape plots rose from 27% in rape crop fields and 33% in the narrow field margins to 49% in large old fallows (Fig. 1B). This experimental evidence was supported by samples from rape crop fields. In the center of rape crop fields, adjacent large fallows had a stronger effect on parasitism than field margins and cereal fields, but at the edge of the rape crop fields, parasitism was not influenced by the type of adjacent vegetation (Fig. 1C). Accordingly, the age and size of noncrop area were the two major factors found to enhance the efficiency of biological control of the rape pollen beetle on a local and medium scale, and these findings were supported by the landscape results.

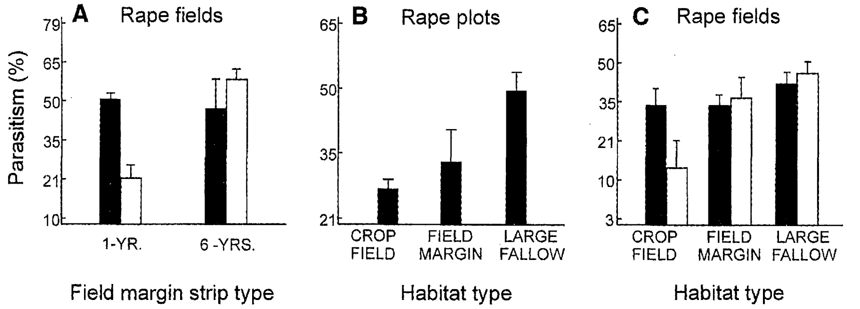

Fig. 1. Parasitism of rape pollen beetle (*M. aeneus*) by *T. heterocerus*, *P. interstitialis*, and *P. morionellus* on oilseed rape (*Brassica napus*) in different types of habitats {arithmetic means ± SE are given, arcsine[(–transformed percentages)$^{1/2}$]}. In rape fields, parasitism near the edge (filled columns) and in the center (open columns) of the fields is given. (**A**) Parasitism in winter rape fields adjacent to different field margin strips (i) near the edge (1 m from the edge) and (ii) in the center (10 to 12 m from the edge) of the fields. Two-way nested analysis of variance (ANOVA): parasitism (%) versus field margin strip type (1-year/6-years: $F = 5.77$, $P = 0.02$, $N = 20$) and sampling site (edge/center: $F = 14.38$, $P = 0.0005$, $N = 40$). *Phradis interstitialis* (on average 4.6% parasitism) was of minor importance in this study. (**B**) Parasitism in experimental summer rape plots established within winter rape crop fields ("crop field," $N = 4$); small (3-m-wide) 6-year-old field margins strips ("field margin," $N = 4$) with naturally developed vegetation; and 6-year-old large fallows ("large fallow", >1 ha, $N = 4$) with naturally developed vegetation. One-way ANOVA: $F = 5.23$, $P = 0.03$, $N = 12$. (**C**) Parasitism in winter rape fields adjacent to cereal crop fields ("crop field," $N = 5$, was barley in the study year and wheat in the year before); small (3-m-wide) old field margins strips ("field margin," $N = 5$) with naturally developed vegetation; and large old fallows ("large fallow," >1 ha, $N = 6$) with naturally developed vegetation. Two-way nested ANOVA: parasitism (%) versus habitat (crop field/field margin/large fallow: $F = 5.75$, $P = 0.009$, $N = 16$) and sampling site (edge/center: $F = 2.19$, $P = 0.11$, $N = 32$).

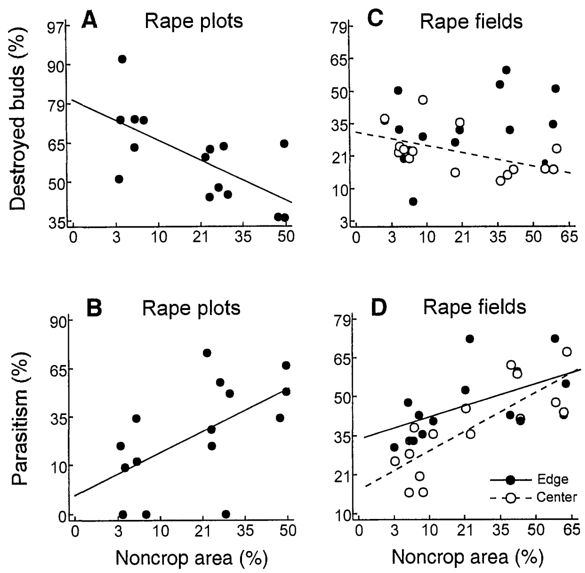

Fig. 2. Plant damage caused by rape pollen beetle (*M. aeneus*) and parasitism of rape pollen beetle by *T. heterocerus*, *P. interstitialis*, and *P. morionellus* on oilseed rape (*B. napus*) in relation to the percentage of noncrop area in agricultural landscapes {arcsine[(–transformed percentages)$^{1/2}$]}. (**A**) Percent destroyed buds in experimental summer rape plots in relation to the percentage of noncrop area. $Y = 70.8 - 0.51X$, $F = 10.06$, $P = 0.007$, $R^2 = 0.44$, $N = 15$. (**B**) Percent parasitism in experimental summer rape plots ($N = 15$) in relation to the percentage of noncrop area. $Y = 7.75 + 0.87X$; $F = 6.51$, $P = 0.02$, $R^2 = 0.33$, $N = 15$. (**C**) Percent destroyed buds in winter rape fields in relation to the percentage of noncrop area. Mean percentages per landscape ($N = 15$) from 26 winter rape fields are given. Edge of the fields: P = not significant; center of the fields: $Y = 37.2 - 0.21X$; $F = 4.85$, $P = 0.046$, $R^2 = 0.27$, $N = 15$. (**D**) Percentage parasitism in winter rape fields in relation to the percentage of noncrop area. Mean percentages per landscape ($N = 15$) from 26 winter rape crop fields are given. Edge of the fields: $Y = 39.4 + 0.28X$; $F = 5.86$, $P = 0.03$, $R^2 = 0.31$, $N = 15$; center of the fields: $Y = 27.1 + 0.5X$; $F = 21.1$, $P = 0.0005$, $R^2 = 0.62$, $N = 15$. The intercepts of these regression lines are significantly different ($F = 4.47$, $P = 0.04$), whereas the slopes do not differ ($F = 1.89$, $P = 0.18$).

Structural simplicity in agricultural landscapes was correlated with large amounts of plant damage caused by the pollen beetle and small amounts of larval mortality caused by parasitism. The percentage of destroyed buds increased (Fig. 2A) and pollen beetle mortality from parasitism decreased (Fig. 2B) as the percentage of noncrop area in the landscape decreased. Rape crop fields showed similar relations. The percentage of destroyed buds was negatively correlated with the percentage of noncrop area (Fig. 2C), whereas the rate of parasitism was positively correlated (Fig. 2D).

Parasitism by each of the two main parasitoid species increased significantly with landscape heterogeneity. In the center of rape crop fields, the rate of parasitism by *T. heterocerus* increased linearly without any evidence of a threshold from on average 6% onward to 38%, and the rate of parasitism by *P. interstitialis* increased asymptotically from 0% to values of 40% (*14*). Rates of parasitism were related neither to host density nor to the percentage of rape fields per landscape. The latter was also not related to the percentage of destroyed buds, contradicting common expectations from plant protection professionals, that landscapes with large areas of rape crop fields should suffer from larger pest populations (*15*).

Landscape complexity or the percentage of noncrop area was closely related with further landscape characteristics (*9*). Isolation of a sampling site from noncrop area decreased and habitat-type diversity increased with the percentage of noncrop area ($R^2 = 0.91$ and $R^2 = 0.87$, $P < 0.001$, $N = 15$).

Our results show the importance of landscape structure for local interactions, thereby explaining why the same experimental design may result in a positive or negative effect depending on the type of landscape. Significant differences between the edge and the center of rape fields (Fig. 1A) could only be found in structurally simple but not in structurally complex landscapes (Fig. 2D), because high percentages of noncrop area obviously enhanced parasitoid populations, thereby masking these effects. When the noncrop area dropped below a value of about 20%, the percentage of parasitism was observed to drop below the threshold value of 32 to 36%, below which a success in classical biological control has never been found (*16*).

These results provide evidence that complex landscapes with a high density and connectivity of uncultivated, perennial habitats may enhance populations of natural enemies, which immigrate into neighboring annual crop fields, attack pest insects, and contribute significantly to the reduction of pest populations below an economic threshold. Nonetheless, because of the presumably very different responses of different organisms to landscape structure (*6*), generalizations for the design and management of agricultural landscapes are still a matter for future research.

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7. Field margin strips were 3 m wide, about 100 m long, and either 1 year old or 6 years old. The 1-year-old field margin strips included naturally developed vegetation ($N = 4$), sown *Phacelia tanacetifolia* strips ($N = 4$), sown wildflower mixtures of 19 species ($N = 4$), and sown (monoculture) wheat ($N = 4$). The six-year-old field margin strips ($N = 4$) were naturally developed. Each of four studied rape fields was surrounded by all five types of field margins in the same structurally simple landscape.
8. Experimental summer rape plots (2 m^2 of sown rape plots; $N = 12$) were placed in three types of habitats: (i) large fallows (>1 ha, 6 years old with naturally developed vegetation) and (ii) small (3-m-wide) field margin strips (6 years old with naturally developed vegetation), and (iii) monocultural rape fields as the control (Fig. 1B). In addition, we also examined the effects of these habitat types on winter rape crop fields ($N = 16$) (Fig. 1C).
9. Landscape structure was estimated in 15 circular landscape profiles with a diameter of 1.5 km by using aerial photographs and intensive field inspections. The area of each habitat type was measured. Noncrop area includes all uncultivated and perennial habitats such as fallows, field margins, grassland, hedges, and woods. The diversity of habitat types was calculated following Shannon's index. The isolation of a sampling site *i* from surrounding noncrop area (indexed by *j*) was measured indirectly by a negative exponential weighting function, $Isolation_i = -\Sigma\,(e^{-Distance} \times non\text{-}crop\ area_j)/\Sigma\, e^{-Distance}$ [see, for example, S. Eber, R. Brandl, *J. Anim. Ecol.* **65**, 621 (1996)].
10. Parasitism was studied during the full-flowering period of rape in May (unsprayed winter rape fields) and June (experimental summer rape plots). All summer rape flowers of five randomly selected plants were collected in each of the 12 experimental plots in different habitats (Fig. 1B) and in each of the 15 experimental plots in different landscapes (Fig. 2B). In each landscape, plots out of two pots (volume, 30 liters; five plants per pot) were established in old field margin strips adjacent to cereal fields. Winter rape flowers were sampled near the edge (1 m into the field) and toward the center of the fields (10 to 12 m into the field): (i) 20 independent samples (each sample with two subsamples of 40 flowering shoots) in winter rape fields adjacent to five different types of field margin strips with four replicates each (Fig. 1A); (ii) 16 independent samples (each with two subsamples comprising all flowers from 0.25 m^2) in winter rape fields adjacent to three different habitat types with five to six replicates each (Fig. 1C); and (iii) 15 independent samples (each with two to four subsamples comprising all flowers from 0.25m^2) in the 15 landscapes (Fig. 2D). Rates of parasitism were measured by dissection of last instar larvae of rape pollen beetle. Plant damage caused by feeding of adult pollen beetles was assessed by the number of destroyed buds, which did not develop into pods and appeared as podless stalks (Fig. 2, A and C).
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13. Our experiments also showed that pollen and nectar resources as well as alternative hosts had no significant effect on parasitoid enhancement, although flower availability greatly differed between field margin types, from the sowings with wheat (as a control) to sowings with species-rich wildflower mixtures.
14. Rates of parasitism (Y) were correlated with the percentage of noncrop area (X) in both parasitoid species. *Tersilochus heterocerus*: $Y = 4.5 + 0.46X$, $F = 5.18$, $P = 0.04$, coefficient of determination $R^2 = 0.285$, $N = 15$ (field edge); and $Y = 6.7 + 0.54X$, $F = 10.2$, $P = 0.008$, $R^2 = 0.44$, $N = 15$ (field center). *Phradis intersitialis*: mean rate = 32.7%, P = not significant (field edge); and $Y = 1/(0.02 + 0.38/X)$, $F = 10.01$, $P = 0.007$, $R^2 = 0.44$, $N = 15$ (field center). High rates of parasitism in complex landscapes were also related to high rates of multiparasitism (Y). Attack of one host larvae by the two parasitoid species at the same time did significantly increase with noncrop area (X), on average from 2 to 27% ($Y = 4.5 + 0.46X$, $F = 15.4$, $P = 0.002$, $R^2 = 0.54$, $N = 15$) at the edge, and from 2 to 32% ($Y = 2.4 + 0.4X$, $F = 32.3$, $P < 0.001$, $R^2 = 0.73$, $N = 15$) in the center of the fields.
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Signaling from Rho to the Actin Cytoskeleton Through Protein Kinases ROCK and LIM-kinase

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The actin cytoskeleton undergoes extensive remodeling during cell morphogenesis and motility. The small guanosine triphosphatase Rho regulates such remodeling, but the underlying mechanisms of this regulation remain unclear. Cofilin exhibits actin-depolymerizing activity that is inhibited as a result of its phosphorylation by LIM-kinase. Cofilin was phosphorylated in N1E-115 neuroblastoma cells during lysophosphatidic acid–induced, Rho-mediated neurite retraction. This phosphorylation was sensitive to Y-27632, a specific inhibitor of the Rho-associated kinase ROCK. ROCK, which is a downstream effector of Rho, did not phosphorylate cofilin directly but phosphorylated LIM-kinase, which in turn was activated to phosphorylate cofilin. Overexpression of LIM-kinase in HeLa cells induced the formation of actin stress fibers in a Y-27632–sensitive manner. These results indicate that phosphorylation of LIM-kinase by ROCK and consequently increased phosphorylation of cofilin by LIM-kinase contribute to Rho-induced reorganization of the actin cytoskeleton.

During cell morphogenesis and motility, cells undergo extensive remodeling of the actin cytoskeleton, a phenomenon that is mediated by various actin-binding proteins (*1*). Such remodeling is often triggered by extracellular stimuli, but the signaling pathways to actin-binding proteins remain largely uncharacterized. The small guanosine triphosphatase (GTPase) Rho is converted from the inactive, guanosine diphosphate–bound form to the active, GTP-bound form in response to stimuli such as serum and lysophosphatidic acid (LPA), and induces various morphological events such as cell adhesion and motility (*2*). Among Rho effectors isolated, the ROCK family of Rho-associated serine-threonine protein kinases is implicated in Rho-mediated cell adhesion and smooth muscle contraction (*2*). These kinases phosphorylate myosin light chain (MLC) phosphatase and inhibit its activity (*3*); this inhibition, in turn, results in an increase in MLC phosphorylation and, consequently, increases actomyosin-based contractility, events that contribute to Rho-mediated stress fiber formation and smooth mus-

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