proportions.

Cloned srRNA genes from *Symbiodinium A*, *B* and *C* were used as standards[7]. They were amplified singly and from defined mixtures of two (Fig. 2b) or three (Fig. 4c) types[7] to assign field samples to classes of symbiont relative abundance by visual comparison (Fig. 2a, b). To validate this procedure, approximately equal numbers of *A*, *B* and *C* cells, from three natural isolates of each type, were mixed in pairwise combinations and analysed. The results implied that *Symbiodinium B* and *C* yield (on a per-cell basis) equal signals, whereas *A* yields about twice that amount. Standard mixtures of cloned genes were adjusted accordingly.

Symbiont densities and chlorophyll contents (Fig. 4d, e) were determined from haemacytometer counts (8 replicate grids per sample) and spectrophotometrically from methanol extracts[29], respectively. These symbionts were isolated quickly (with minimal washing) from frozen samples at 4 °C under dim light. Symbiont genotypes, numbers and chlorophyll contents were obtained from subsamples of each isolate.

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Correspondence and requests for materials should be addressed to R.R. at the University of Guam Mante Laboratory (e-mail: rrowan@uog9.uog.edu).

A polymorphism maintained by opposite patterns of parasitism and predation

John E. Losey, Anthony R. Ives, Jason Harmon, Ford Ballantyne & Carrie Brown

Department of Zoology, University of Wisconsin at Madison, Madison, Wisconsin 53706, USA

Although polymorphism is a widespread phenomenon that has been recognized for nearly two centuries, the basic mechanisms maintaining most polymorphisms in nature are unknown[1,2]. We present evidence that a polymorphism can be maintained exclusively by balanced selection from two predatory species. For field and laboratory experiments, we used the pea aphid, *Acyrthosiphon pisum*, which occurs as 'green' and 'red' colour morphs, and two species that attack pea aphids, the parasitoid *Aphidius ervi* and the predator *Coccinella septempunctata*. We found that when parasitism rates in the field were high relative to predation rates, the proportion of red morphs increased relative to green morphs, whereas the converse was true when predation rates were high relative to parasitism rates. Detailed laboratory and field studies confirmed that green morphs suffer higher rates of parasitism than red morphs, whereas red morphs are more likely to be preyed on by predators than green morphs are. We present a mathematical model that demonstrates that biased density-dependent parasitism and/or predation on different morphs is sufficient to maintain the colour polymorphism in the population. Our findings support an important role for predation in the maintenance of genetic diversity.

Aphids occur in a range of colour morphs that can differ in growth rates, host range, defensive behaviour, and susceptibility to parasitism[3–6]. Pea aphids, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), in south-central Wisconsin occur as two colour morphs, green and red. The colour morphs remain distinct through the summer months because the aphids reproduce parthenogenetically. Pea aphids experience high levels of parasitism by the wasp *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) and heavy predation by several predators, including ladybird beetles, especially *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *A. ervi* is a 'parasitoid' that attacks aphids by inserting an egg through the aphid's cuticle; the developing wasp larva feeds on and eventually kills the aphid[7]. Both the parasitoid and the predator can have a major impact on pea aphid populations[8,9] and hence may be important selective agents. Although all three species are introductions to the Neartic, they have a long evolutionary history in their common Paleartic home range, where both aphid colour morphs also coexist[10–12].

In field populations, we found that the relative level of parasitism and predation had a significant effect on aphid colour morph composition. Specifically, the proportion of red morphs increased following relatively high parasitism and decreased following relatively high predation (Fig. 1), implying balancing selection by parasitism and predation. This balanced parasitism/predation hypothesis was supported by our further studies demonstrating directly that parasitism by *A. ervi* is heavier on green morphs, whereas predation by *C. septempunctata* is heavier on red morphs. The parasitism rate on green morphs in the field (53%) was significantly higher than on the red morph (42%) ($P < 0.001$). Significantly higher parasitism rates on a green morph over a red morph have also been reported for the alfalfa aphid, *Macrosiphon creelii* Davis[13]. Another study found higher parasitism on red than on green morphs of *A. pisum*[4]: the difference between this result and

ours may be due to differences in experimental protocol (that is, laboratory rather than field) or differences in the aphid or parasitoid strains used.

The high mobility of ladybird beetles made it impossible for us to measure predation in the field. However, we measured predation rates by *C. septempunctata* on caged plants in a greenhouse, and the predation rate on red morphs (0.91 ± 0.08 aphids eaten per hour) significantly exceeded that on green morphs (0.73 ± 0.06 aphids eaten per hour) ($P < 0.04$). Thus the observed pattern of parasitism and predation supports the hypothesis that the polymorphism is maintained by a balance of these two sources of mortality.

In all previous studies in which predation is a selective force maintaining a polymorphism, susceptibility to predation of some morphs is balanced against some other trait which makes susceptible morphs more competitive in the absence of predation. We found no evidence for this type of balance. Reproductive rates of the two morphs were not significantly different ($P = 0.437$). Thus, a hypothesis that greater parasitism or predation rates on one morph were balanced by greater reproductive rates is not supported. Furthermore, the propensity to drop from a plant when confronted with a foraging predator, an important aphid defensive behaviour, did not differ significantly between the two morphs ($P = 0.226$; see ref. 14 for methods). Although there may be other more subtle differences in the biology of the two colour morphs[12], the evidence implicates balanced parasitism and predation as the factors that maintain the colour polymorphism.

The differential susceptibility of colour morphs suggested that the parasitoid and predator may be using prey colour as a foraging cue, and both *A. ervi* and *C. septempunctata* have been shown to use visual cues to locate prey[15,16]. To investigate the use of colour cues by *C. septempunctata*, we measured predation rates on both morphs in red, green or white containers. In green containers, predation by *C. septempunctata* was higher on the red morph, whereas in red containers predation was higher on the green morph. There was no difference in predation between the morphs in white containers (Table 1). These results indicate that red morphs are more susceptible on green plants because they are more visible.

The explanation for higher *A. ervi* parasitism on the green morph is not as obvious, as the green morph should be relatively more cryptic visually than the red morph on green plants. In an experiment similar to that on *C. septempunctata*, *A. ervi* showed no effect of container colour on relative parasitism rates on the two morphs. However, neither visual nor olfactory discrimination of the aphid morphs by the parasitoids can be completely ruled out by these experiments. In addition, differential encapsulation of parasitoid eggs is a possible mechanism for different parasitism rates on the morphs, which does not involve discrimination by the parasitoid[4]. However, we found no evidence of encapsulation in dissections of over 2,000 aphids containing eggs. Despite the clear evidence for higher parasitism rates on green morphs in the field, the mechanism for this pattern is unknown.

To examine the consequences of morph-biased parasitism and predation, we constructed a simple model of aphid morph–parasitoid–predator population dynamics (Box 1). A central assumption of the model is that one or both of the predatory species will exert density-dependent mortality on the aphids. As pea aphids make up the vast majority of *A. ervi* hosts in agricultural systems, *A. ervi* population dynamics are coupled to those of its hosts, so density dependence can be assumed. Although *C. septempunctata* is a generalist predator with a wide host range, it exhibits aphid-

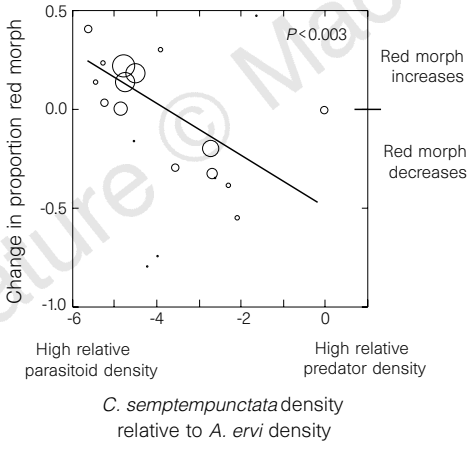

Figure 1 Change in proportion of red morphs in the aphid population between successive field samples versus the relative predation pressure. The sizes of the circles are proportional to weightings based on sample sizes. The regression is statistically significant at the $P < 0.003$ level ($n = 20$).

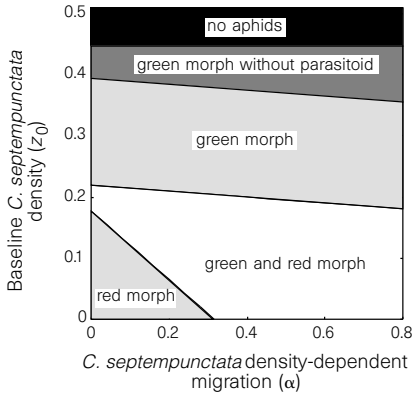

Figure 2 Theoretical conditions leading to coexistence of green and red aphid morphs. Combinations of z_0 and α are shown that satisfy the condition for coexistence of colour morphs. Although details depend on the particular equations and values of the parameters used (Box 1), the qualitative structure of the figure–particularly the existence of a region of morph coexistence–is the same for all biologically plausible models.

Table 1 Numbers of morphs eaten on different backgrounds

Background	Red morphs eaten	Green morphs eaten	*P*
Green	2.10 ± 0.31	1.45 ± 0.31	0.014
Red	0.80 ± 0.16	1.45 ± 0.26	0.014
White	1.40 ± 0.24	1.55 ± 0.30	0.563

The numbers of green and red pea aphid morphs (mean ± s.e.) eaten by *C. septempunctata* on different coloured backgrounds are shown.

Table 2 Distribution of *C. septempunctata* relative to aphid density

Source*	df	F-ratio	*P*
Aphid density	1	5.56	0.025
date	5	3.23	0.019
error	30	0.488	

* The estimate of the slope (α) is 0.60, and the intercept (z_0) is estimated as the average for all dates; $z_0 = 0.11$.

Box 1 General mathematical model

A model to investigate the potential for balanced predation and parasitism to maintain two colour morphs can be derived as follows. Let x_1 and x_2 denote the densities of green and red morphs, and y and $z[x_1,x_2]$ the densities of *A. ervi* and *C. septempunctata*, respectively; density-dependent migration makes $z[x_1,x_2]$ depend on the number of green and red morphs in the field. The population dynamics may be described by

$$\frac{dx_1}{dt} = x_1 f[x_1 + x_2, (1+p)y + bz[x_1,x_2]]$$

$$\frac{dx_2}{dt} = x_2 f[x_1 + x_2, y + (1+q)bz[x_1,x_2]]$$

$$\frac{dy}{dt} = yg[(1+p)x_1 + x_2 + x_2, y]$$

where f and g are functions for the per capita population growth rates of aphids and *A. ervi*, respectively. We assume that interactions between aphid morphs are symmetrical, so f depends on the sum $x_1 + x_2$. The higher parasitism rate on the green morph is modelled by augmenting the parasitism rate by a fraction p above that for the red morph, and predation by *C. septempunctata* on the red morph is augmented by a fraction q. Finally, b is the predation rate by *C. septempunctata* relative to the parasitism rate by *A. ervi*.

For any biologically plausible functions f and g, there is always a range of values of p and q that produce coexistence of green and red morphs. For functions $f[x,y]$ and $g[x,y]$, let x and y represent aphid density and combined predator densities. It is biologically reasonable to assume that $df/dx \leqslant 0$, $df/dy < 0$, $dg/dx \geqslant 0$, and $dg/dy \leqslant 0$. Further, let x_1^* and y_1^* denote the aphid and *A. ervi* densities at equilibrium in the absence of the red morph, and define x_2^* and y_2^* similarly when the green morph is absent. Both morphs will coexist whenever $y_1^* > q/p\,b\,z[x_1^*]$ and $q/p\,b\,z[x_2^*] > y_2^*$. As *A. ervi* has a higher parasitism rate on the green morph, y_1^* will always be greater than y_2^*. Furthermore, the higher parasitism rate on green morphs drives down the morph density, so $x_1^* \leqslant x_2^*$ and consequently $z[x_1^*] \leqslant z[x_2^*]$. Therefore, it is always possible to select values of p and q that produce coexistence. (Detailed mathematical proofs will be provided on request.) Note that if *C. septempunctata* did not show aphid-density-dependent migration ($z[y_1^*] = z[y_2^*]$) and *A. ervi* was not a specialist ($y_1^* = y_2^*$), then no coexistence is possible.

The particular equations used for our numerical example (Fig. 2) are

$$\frac{dx_1}{dt} = rx_1\left(1 - \frac{x_1 + x_2}{K}\right) - a(1+p)x_1 y - bx_1 z[x_1,x_2]$$

$$\frac{dx_2}{dt} = rx_2\left(1 - \frac{x_1 + x_2}{K}\right) - ax_2 y - (1+q)bx_2 z[x_1,x_2]$$

$$\frac{dy}{dt} = cy((1+p)x_1 + x_2) - dy$$

which employ logistic aphid population growth, a linear per capita parasitism rate by *A. ervi*, a, and a linear predation rate by *C. septempunctata*, b. We assume that the number of *C. septempunctata* in a field is given by $\sqrt{z[x_1,x_2]} = z_0 + \alpha(x_1 + (1+q)x_2)$. Differential parasitism and predation on the two morphs are included as $(1+p) = (0.53/0.42) = 1.26$ and $(1+q) = (0.91/0.74) = 1.23$ obtained from our experiments. The aphid intrinsic rate of increase, r, is estimated as 0.2 day^{-1}. The parameter K acts as a scaling parameter of aphid density and was arbitrarily set to 1. We assume that per capita parasitism and predation rates are the same, $b = 1$. Other parameter values are $a = 1$, $c = 0.5$ and $d = 0.25$. □

density-dependent migration[9,17–19]. We confirmed the importance of aphid-density-dependence in our system by showing that there were statistically significantly more *C. septempunctata* found in fields with more aphids (Table 2).

The model shows that the coexistence of the two colour morphs is facilitated by both density-dependent parasitism resulting from the coupling of host and parasitoid population dynamics, and density-dependent migration that leads to greater predator densities in fields with high aphid abundance. Although the density dependence of *A. ervi* that results from its population dynamics being coupled to those of the aphids promotes morph coexistence, even in the absence of *C. septempunctata* density dependence, the potential for coexistence is greatly enhanced if the predator exhibits density dependence as well (Fig. 2). Thus the maintenance of the colour polymorphism is not simply a matter of two predatory species preferentially attacking alternate morphs: it also requires density-dependent mortality from the parasitoid and/or predator.

The discovery that balancing parasitism/predation can maintain a polymorphism indicates that other unexplained polymorphisms may be maintained by balanced density-dependent mortality from predators, parasitoids and/or pathogens, as almost all organisms are killed by a complex of predatory species. Determining an underlying selective mechanism of polymorphism maintenance bears directly on the long-standing debate on whether the bulk of polymorphisms should be classified 'adaptive' or 'neutral'[20,21]. Our results also have implications for the conservation of biodiversity, given the importance of preserving genetic diversity within insect species in addition to conserving the species themselves[22–24]. An understanding of the mechanisms that maintain genetic diversity will enable conservation programs to be more effective. □

Methods

Assessment of parasitoid, predator, and aphid densities in the field. We estimated the density of the parasitoid, the predator, and the two aphid morphs by sampling 12 alfalfa fields roughly every 6 days throughout the summer of 1996. In each field, aphid density and colour were recorded for 100 stems in eight locations. Twelve three-minute walking scan samples of parasitized aphids and *C. septempunctata* adults were also made throughout the field. Parasitoid counts were limited to 'mummified' pea aphids which are immobile, skeletonized aphids containing one parasitoid larva or pupa. Sampling dates followed by an alfalfa harvest and fields without aphids were excluded from the analysis.

Impact of parasitoids and predators on relative proportions of colour morphs. We measured the impact of parasitoids and predators on the relative proportions of colour morphs by regressing the change in proportion of red morphs in the aphid population between successive field samples versus the relative predation pressure. The relative predation pressure was measured as $\log[(C7_{t-1} + 1)/(C7_{t-1} + m_t)]$, where $C7_{t-1}$ is the number of *C. septempunctata* observed during 12 three-minute scan samples in a field in week $t - 1$, and m_t is the number of mummies observed in the field during scan samples in week t. We used m_t as a measure of parasitism in week $t - 1$ because mummies form roughly one week after parasitism. The regression of $\Delta r = r_t - r_{t-1} = \text{constant} + \text{slope}^* \log[(C7_{t-1} + 1)/(C7_{t-1} + m_1)]$ was done while weighting by the square-root of the total number of aphids counted in either week $t - 1$ or week t, whichever had the fewest aphids. Weighted regression was necessary to correct for higher variance in Δr created when the number of aphids counted per field was small.

***C. septempunctata* density dependence.** We tested for aphid-density-dependent migration by *C. septempunctata* by regressing aphid density (at each sample date) versus *C. septempunctata* density. The regression model was $\sqrt{C7} = z_0\,^*\text{date} + \alpha^* A/\bar{A}$, where $C7$ is the number of *C. septempunctata* observed per field in 12 three-minute scan samples, A is the number of aphids per 800 alfalfa stems, $\bar{A}$ is the mean number of aphids per 800 stems for all fields, and 'date' is a categorical variable for the date of the sample. Only those dates on which *C. septempunctata* were found in two or more fields are included.

Assessing parasitism rate. To estimate parasitism rates, we sampled five

alfalfa fields on five dates in May, June and August 1996, and dissected a total of 643 aphids for larval *A. ervi*. Data were analysed with χ^2 test, blocked by field and date.

Susceptibility of colour morphs to *C. septempunctata* on plants. We measured the susceptibility of the two aphid colour morphs to predation by allowing a single adult *C. septempunctata* to forage on caged alfalfa plants with 15 adults of each morph for 4 h; the experiment was replicated 27 times. After removing the predator from each plant, the number of remaining aphids of each morph was recorded and subtracted from 15 to determine the number consumed. The data were analysed with a 2-tailed, paired *t*-test.

Use of colour cues by *C. septempunctata*. We investigated the use of colour cues by *C. septempunctata* by allowing single adult beetles to forage for aphids of both colour morphs in red, green and white containers. To maximize our ability to detect differences in predation rates between morphs, we designed the experiment as a split-plot, with background as the whole plot and aphid colour morph as the split plots. Each arena had a single background colour and 5 similar-sized (third or fourth instar) aphids of each colour morph. Predators foraged for 30 min, and at the end of the experiment the number of remaining aphids of each colour morph was recorded and subtracted from 5 to determine the number consumed. All predators foraged actively and consumed at least one aphid. Each treatment was replicated 20 times. Data were analysed as a split-plot analysis of variance.

Comparison of reproductive rate of the colour morphs. The reproductive rates of the aphid colour morphs were compared by pairing 10 of each morph on individual caged alfalfa plants and allowing them to reproduce for 14 days. At the end of the experiment, all aphids of each morph was recorded. The experiment was started with 14 plants, but 2 plants were severely wilted after two weeks so these were excluded from the analysis. Data were analysed with a 2-tailed paired *t*-test.

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Correspondence and requests for materials should be addressed to J.E.L. (e-mail: jlosey@facstaff.wisc.edu).

Multistability of cognitive maps in the hippocampus of old rats

Carol A. Barnes*†, Matthew S. Suster*, Jiemin Shen* & Bruce L. McNaughton*‡

** Departments of Psychology and Neurology, and ‡Departments of Psychology and Physiology, † Arizona Research Laboratories Division of Neural Systems, Memory and Aging, University of Arizona, Life Sciences North Building, Room 384, Tucson, Arizona 85724, USA*

Hippocampal neurons provide a population code for location[1]. In young rats, environments are reliably 'mapped' by groups of neurons that have firing locations ('place fields'[2]) that can be stable for several months[3]. Old animals exhibit deficits in spatial memory, raising the question of whether the quality or stability of their hippocampal 'cognitive maps'[4] is altered. By recording from large groups of neurons, we observed the hippocampal spatial code to be multistable. In young rats, the place field maps were reliable both within and between episodes in a familiar environment. In old rats, place field maps were accurate and stable during an episode, but frequently exhibited complete rearrangements between episodes. In a spatial memory task, both young and old rats exhibited bimodal performance, consistent with map multistability early in training. However, the performance of young rats became almost unimodal with further training, whereas that of old rats remained markedly bimodal. The multistability of the hippocampal map provides an insight into the dynamics of neural coding in high-level cortical structures and their changes during ageing, and may provide an explanation for the frequent failure of place recognition in elderly humans.

Spatial memory is deficient in healthy elderly humans[5], non-human primates[6] and rodents[7]. The hippocampus is important for spatial learning[4], and exhibits complex, region-selective changes in its anatomical connectivity, synaptic physiology and neurochemical organization during ageing[8–10], including a decline in its ability to maintain synaptic long-term potentiation (LTP)[7–11]. Paradoxically, however, there are only small changes in the spatial information conveyed by hippocampal pyramidal neurons in aged animals[12–14].

How is this apparent preservation of spatial signalling, despite substantial alterations in cellular physiology and behaviour, to be explained? Place fields can appear in complete darkness during the first trial in an environment and remain unchanged in subsequent illumination[15], suggesting that place-field interrelationships can be prespecified in the synaptic matrix[16], rather than learned or imposed by external stimuli, as earlier theories had suggested[17–20]. Current evidence indicates that self-motion signals, rather than relationships among landmarks *per se*, are used to specify relative position on a place-field map[4,16,21]. However, landmark information seems to become bound to map locations through associative learning, and can subsequently provide position fixes that correct for the inevitable drift error in such a self-motion-based navigational system[21,22]. Associative binding of landmark information to preconfigured maps would also enable the recall of a consistent map for a previously visited environment[16]. If place-field maps depend primarily on pre-existing weights within the network but landmark binding depends on LTP of external inputs, then the LTP deficit that occurs in old rats[7,10,11] might result in the failure to select a consistent map for a given environment, even though the place fields themselves would appear relatively normal.

Multiple CA1 pyramidal cells were monitored simultaneously (number of cells per session (mean ± s.e.m.): young, 34 ± 13; old, 37 ± 16) during the course of two consecutive episodes of running