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Evolutionarily stable investment in secondary defences

M. BROOM,*† M. P. SPEED‡ and G. D. RUXTON§

**Centre for Statistics and Stochastic Modelling, Department of Mathematics, University of Sussex, Brighton BN1 9RF, UK, ‡School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB, UK, and §Division of Environmental & Evolutionary Biology, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK*

Summary

1. Previous workers have suggested that the evolutionarily stable strategy (ESS) for investment in antipredator defences, such as toxins, will critically depend on the nature of expression of the defence. Specifically, it has been suggested that if the different levels of a defence are best described as a continuous variable, then this will lead to pure ESSs with all individuals in a population adopting similar defence levels; whereas defences that can only take on discrete levels will lead to mixed ESSs (featuring variation in defence within the population).

2. Our principal aim is to determine the validity of these viewpoints, and examine how the pure and mixed strategies predicted by the two types of defences can be reconciled with practical and philosophical difficulties in defining any given defence unambiguously as continuous or discrete.

3. We present the first model of a continuously varying defence that is solved explicitly for evolutionarily stable strategies.

4. We are able to demonstrate analytically, that the model always has a unique ESS, which is always pure. This strategy may involve all members of the population adopting no defence, or all members of the population making the same non-zero investment in defence.

5. We then modify our model to restrict the defence to a number of discrete levels and demonstrate that the unique ESS in this case can be either pure or mixed. We further argue that the mixed ESS can be a combination of no more than two defence levels, and the two levels in a mixed ESS must be nearest neighbour levels in an ordered list of the levels that the defence can take.

6. This, in turn, means that the mixed ESS will be practically identical to a pure ESS if the discrete defence is fine-grained.

Key-words: Automimicry, Evolutionarily Stable Strategy, levels of defence, mixed strategy, toxins

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Introduction

Many prey animals in terrestrial and aquatic environments have defences, such as spines, stings, toxins and sticky secretions, that come into play after a predator has singled out a particular prey individual for attack; these are often called secondary defences (Edmunds 1974; Whitman, Blum & Alsop 1990). The level of secondary defences often differs among individuals in the same prey population (review in Ruxton, Sherratt & Speed 2004). For example, in some populations a fraction of the prey may lack defences altogether (Brower, Pough & Meck 1970); while in other populations all individuals are defended to some extent but there may be considerable variation between individuals in the levels of this defence, and perhaps the precise types of toxins present (Bowers 1992; Holloway *et al*. 1991). Though well known, the existence of intraspecific variation in secondary defences has received remarkably little attention from evolutionary biologists. Instead much greater attention has been given to the evolution of signalling traits associated with secondary defences, particularly aposematism and mimicry.

This neglect is misplaced, since, for example, understanding of the evolutionary dynamics of secondary defences should be an essential prerequisite for consideration of signals of those defences. Although optimization theory has been applied to the study of inducible secondary defences (e.g. Clark & Harvell 1992; Frank 1993; Adler & Karban 1994; Tollrian & Harvell 1999), it has rarely been used to examine the evolutionary

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†Author to whom correspondence should be addressed. E-mail: M.Broom@sussex.ac.uk

stability of defences that are expressed permanently. The most important and influential theoretical work on the evolution of such constitutive defences is that of Leimar *et al*. (1986), which considered level of defence to be a continuous trait, in that the level of a given defence could take an infinite number of values varying smoothly over a defined range. Thus level of defence could be well described in the model by a variable taking real number values. Leimar *et al*. (1986) provide quantitative arguments that indicate that any evolutionarily stable strategy (ESS) in their model will always be a pure strategy. That is, at ESS all members of the population will invest equally in defence.

In contrast, Guilford (1988, 1994) and Ruxton *et al*. (2004) suggest that the ESS would be different when costly traits that enhance survival are discontinuous in their expression (i.e. they can take on only a number of discrete values). Specifically, they suggest that in this case the evolution of defence may be characterized by mixed ESSs where conspecifics with different levels of defence coexist at evolutionarily stable frequencies with equal fitness. This conjecture is supported by the quantitative modelling of Till-Bottraud & Gouyon (1992) and Speed *et al*. (2005). Hence, there is a body of work which cumulatively suggests that the nature of the variation in defence shown by a population should be fundamentally different depending on whether the defence can be expressed across a continuous range of values or only in a discrete set of levels. This presents both a practical and philosophical challenge, since definitive classification of a defence as discrete or continuous is difficult, not least since the expression of traits is a function of both an organism's genotype (and gene changes are discrete actions) and the environment (generally categorized as continuously variable). Hence this paper sets out to achieve three main objectives:

1. To explore the generality of the model prediction of Leimar *et al*. (1986) that the predicted ESS of defence should be expected to be a pure strategy in models that represent defence as a continuous trait.
2. To use a development of this model to search for general rules about the nature of the mixed ESSs that have previously been demonstrated for situations where levels of defence are discrete.
3. To understand how the pure and mixed strategies predicted for the two types of defences can be reconciled with practical and philosophical difficulties in defining a given defence unambiguously as discrete or continuous.

The model framework

We restrict our analysis to 'invisible' defences that cannot be evaluated prior to an attack. Specifically this means internally stored chemical defences (toxins, secretions, etc.) rather than physical defences such as spines. We consider a simple population with discrete generations and asexual reproduction. At the start of each generation, there are N individuals, which must survive for a time T before reproducing. Each individual i is characterized by its defence level D_i. Level of defence influences survivorship in two ways. Firstly, if an individual is attacked by a predator, then its probability of surviving that attack increases with increased investment in defence. Specifically, if individual i is attacked, then its probability of surviving that attack is given by

$$1 - \exp(-sD_i), \qquad \text{eqn 1}$$

for some positive constant s. Secondly, we assume that predators must invest more time recovering from attacking more highly defended individuals. This can be thought of as the time taken to recover from ingesting chemical toxins (this is a common assumption in models of defences and mimicry, e.g. Huheey 1964; Brower *et al*. 1970; Pough *et al*. 1973; Augner & Bernays 1998). Whatever its physiological basis, we use this mechanism to define defence level. Specifically D_i is the time that a predator would have to invest in attacking individual i and recovering from such an attack; during this time it is unable to simultaneously seek further prey to attack. An alternative interpretation is that D is driven psychologically rather than physiologically, and is the time after an aversive experience for which the predator is unwilling to attack other similar looking prey. Whatever its basis, the consequence of this mechanism is that the number of attacks that the prey population faces during a generation (A) will be a declining function of investment in defence by that population. We assume that when predators are actively searching for prey, they encounter them as a Poisson process. The probability that individual i survives to reproduce at the end of the generation is

$$S(D_i) = E[(1 - \exp(-sD_i))^X], \qquad \text{eqn 2}$$

where X is a random variable with mean A/N. Providing predator pressure is sufficiently light that prey are relatively unlikely to be attacked (i.e. A is substantially lower than N), then this is well approximated by

$$S(D_i) \approx 1 - \left(\frac{A\exp(-sD_i)}{N}\right) \qquad \text{eqn 3}$$

Although we require this assumption to provide analytic tractability, it should be noted that from the simulations later in the paper we see that the key predictions of the analytic model developed here are robust against violation of the assumption of light predation pressure. We assume that investment in defence is costly, and that this cost is paid in reduced fecundity of individuals that survive to the end of the generation. Hence, if individual i survives to reproduce, its fecundity is simply

$$f(D_i) = \exp(-cD_i), \qquad \text{eqn 4}$$

for some positive constant c. Combining survival and fecundity gives us the expected fitness of individual i as

$$F_i(D_i) = S(D_i)f(D_i), \qquad \text{eqn 5}$$

which for our chosen functional form reduces to

$$F_i(D_i) = \exp(-cD_i) - \frac{A\exp(-(s+c)D_i)}{N} \qquad \text{eqn 6}$$

The ESS when defence is a continuous variable

We assume that D_i can take any non-negative real number. We wish to find the value of D_i that maximizes F_i. We shall call this value D_o. If we further assume that the population size (N) is sufficiently large that the overall number of attacks on the population (A) is negligibly affected by the specific D-value selected by any one individual, then we can consider that A is effectively independent of D_i and so

$$\frac{dF_i}{dD_i} = -c\exp(-cD_i) + \frac{(s+c)A\exp(-(c+s)D_i)}{N} \qquad \text{eqn 7}$$

which in turn implies that

$$-c\exp(-cD_o) + \frac{(s+c)A\exp(-(c+s)D_o)}{N} = 0 \qquad \text{eqn 8}$$

Removing the common factor of $\exp(-cD_o)$ and rearranging gives

$$D_o = \left(\frac{1}{s}\right)\log\left(\frac{A(s+c)}{Nc}\right). \qquad \text{eqn 9}$$

Differentiating equation 7 again and substituting the result from equation 9, it is clear that the second derivative is negative and thus this is a local maximum. Since, to be biologically plausible we demand that D_o is non-negative, we can see that we find a unique and allowable D_o providing we satisfy the condition

$$\left(\frac{A}{N}\right)\left(1+\frac{s}{c}\right) > 1 \qquad \text{eqn 10}$$

Since we are assuming that A is less than N, this can be interpreted as a requirement that the direct survival benefits of investment in defence (controlled by s) are sufficiently great to compensate for the costs of investment in defence (controlled by c). If we fail to satisfy this condition, then the optimal value of D_i is zero, and no investment in defence is predicted.

Suppose that all individuals in the population choose a given level of defence D^*. If in this population the defence level that maximizes fitness D_o is equal to D^* then, since we have assumed that a population is sufficiently large that the effect of a mutant playing a different strategy is negligible on A, D^* is evolutionarily stable. In fact D^* is clearly the unique ESS, and is given by

$$D^* = \frac{1}{s}\log\left(\frac{(s+c)A(D^*)}{cN}\right) \qquad \text{eqn 11}$$

If however,

$$\left(\frac{A(0)}{N}\right)\left(1+\frac{s}{c}\right) < 1 \qquad \text{eqn 12}$$

where $A(0)$ is the number of attacks when no individuals in the population are defended, then $D^* = 0$ is the unique ESS solution.

In summary, when the level of defence is a continuous variable, then there is always a unique ESS for investment in defence. If equation 12 is satisfied then all individuals should make no investment in defence, otherwise they should all adopt the defence level given by equation 11.

Note that it is possible to think of functional forms for $S(D)$ and $f(D)$ that would generate more than one such ESS; if the probability of survival has more than one local maximum for some A, correspondingly there may be more than one pure ESS. The forms we have chosen are both simple enough to get meaningful analytical results, but flexible enough to account for a variety of biologically plausible situations. In general, it seems clear that the probability of survival should be expected to be an increasing function of defence and fecundity should be a decreasing function of defence. All of our qualitative model predictions will be robust providing the functional forms combine to provide a unique value of defence that maximizes fitness, i.e. a single D_o as obtained in equation 9. For there to be a mixed ESS, we would need a functional form where more than one defence level could yield the same maximum fitness. The most obvious way to obtain a range of defence values with equal fitness would be to have flat parts to the functional forms, such that there are a range of defence levels that have identical costs and benefits. Although it is biologically plausible that there are situations where increased investment in defence brings no benefit (i.e. does not increase changes of survival), it seems biologically unlikely that that this increased investment in defence will come at no extra cost. That is, we expect that in the overwhelming majority of biological cases, increasing levels of defence will be increasingly costly, and so a range of defence levels having equal fitness is unlikely. If these defence levels did not constitute such an interval, such a mixture would be liable to invasion by intermediate defence levels for all but the most unusual functional forms. Thus while it is possible for there to be more than one pure ESS, mixed ESSs are highly unlikely. Further, we consider the possibility of more than one local maxima to be physiologically less likely than the unique optimum condition obtained with our functional forms.

The ESS when defence is a discrete variable

We now assume that the investment in defence of individual i (D_i) can only take a number of discrete values $d_1 < d_2 < d_3 < d_4 \ldots$ Consider a population, a fraction p of which has defence level d_a and a fraction $1 - p$ of which has defence level d_b, where $d_a < d_b$. The number of attacks occurring (A) will be a function of this population mixture, which we shall write as $A = A[pd_a, (1 - p)d_b]$.

We want a condition for the fitness of individuals with investment d_a to be greater than those with fitness d_b. Using equation 6 and defining

$$G(d_a, d_b) = \frac{N(\exp(-cd_a) - \exp(-cd_b))}{\exp(-(s+c)d_a) - \exp(-(s+c)d_b)} \quad \text{eqn 13}$$

it is easy to show that this condition is

$$G(d_a, d_b) > A(pd_a, (1 - p)d_b). \quad \text{eqn 14}$$

Now G is independent of p, whereas the number of attacks (A) always increases with increases to the fraction of prey that have the lower defence level (increasing p). Hence there is either a single critical value of p (p^*) at which the two types have equal fitness, or one type always has higher fitness than the other, regardless of the value of p. That is, we would expect the d_a individuals to have higher fitness (and so increase as a proportion of the population) if

$$A(d_a) < G(d_a, d_b). \quad \text{eqn 15}$$

Conversely, d_b individuals will increase in the population if

$$A(d_b) > G(d_a, d_b) \quad \text{eqn 16}$$

and a stable equilibrium with individuals of both types making up constant (non-zero) proportions of the population through time if

$$A(d_a) > G(d_a, d_b) > A(d_b). \quad \text{eqn 17}$$

It is clear from inspection of equation 6, and the derivative of equation 7, that if we look at fitness as a function of investment in defence, this function has at most one turning point, which is a maximum. Thus if any pair of defence levels are in equilibrium of the type described by equation 17, then every (allowable) defence level between those two levels could invade the population, but no defence values outside their range could. From this, a number of consequences emerge:

1. No collection of three or more defence levels can coexist as an ESS.
2. No pair of non-adjacent defence levels can form an ESS.
3. Any pair of adjacent levels that are in equilibrium, i.e. that satisfy equation 17, form an ESS at the critical mixture value $p = p^*$.
4. There will be at most one pairwise ESS, where the adjacent pair of allowable levels span the predicted pure ESS level of defence if defence were continuous (given by equation 11).
5. If the ESS for the continuous case was that no one should invest in defence then, if only a discrete number of levels are allowable, all individuals should adopt the lowest allowable level of defence.

We now turn to the consideration of pure ESSs. For d_b to be a pure ESS, then a population playing d_b must be able to resist invasion from all other defence levels. From our arguments above, if the population can resist invasion from the defence level immediately below (d_a) and immediately above (d_c), then it can resist all other defence levels. It is easy to see from our definitions above that this occurs providing the following condition is satisfied:

$$G(d_b, d_c) > A(d_b) > G(d_a, d_b). \quad \text{eqn 18}$$

From consideration of equations 17 and 18, it is immediately clear that pure and mixed ESSs cannot coexist. We now consider uniqueness of ESSs more fully.

For a number of discrete defence levels $d_1, d_2, \ldots$ it is easy to see that

$$A(d_1) > A(d_2) > \ldots > A(d_k) > A(d_{k+1}) > \ldots \quad \text{eqn 19}$$

It is also easy to see that

$$G(d_1, d_2) < G(d_2, d_3) < \ldots < G(d_k, d_{k+1}) < G(d_{k+1}, d_{k+2}) < \ldots \quad \text{eqn 20}$$

Since the chain of As is always decreasing with increasing investment in defence (equation 19) and the chain of Gs is always increasing (equation 20), then it is clear that equation 17 or 18 can be satisfied at most once, and so there can only ever be at most one ESS. If the chains cross, then the ESS can be pure or mixed, depending on whichever of equation 17 or 18 is satisfied. If however, $A(d_i)$ is smaller than $G(d_i, d_{i+1})$ for all i-values then pure d_1 is the unique ESS. That is, at the ESS each individual minimizes investment in defence. Finally if $A(d_i)$ is smaller than $G(d_i, d_{i+1})$ for all i, then the highest level of defence is the ESS. Note that for more complex functional forms with more than one turning point, we expect an equivalent argument to be valid at each of these turning points, and so we conjecture that there will be a corresponding discrete ESS for each continuous one. The situation may be more complex if these turning points are close together, or the discrete version is coarse grained.

A simulation model

The analysis of the last section has allowed us to draw a number of general and clear conclusions about the ESS for investment in defence, but our analysis is valid only for situations where A is considerably less than N, that is where individuals have a low risk of being attacked. In order to relax this restriction, we turn from an analytical model to an individual-based simulation model. The rules of the model are exactly like that described in our previous analysis, except that we must now specify a specific function for the predator's

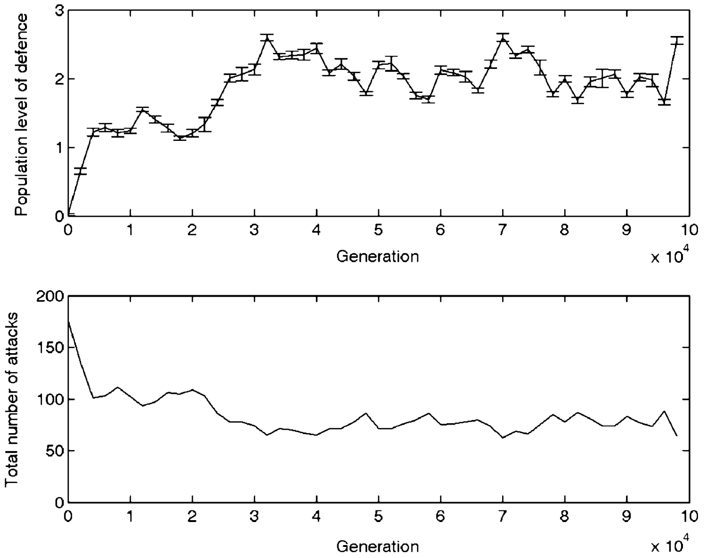

Fig. 1. (a) The mean and the standard deviation (across the population) in level of defence, and (b) the total number of attacks occurring in a generation, both recorded every 2000th generation. Parameter values: $T = 200$, $N_1 = 200$, $\lambda = 0{\cdot}01$, $s = 0{\cdot}12$, $c = 0{\cdot}05$, $\varepsilon = 0{\cdot}01$.

attack rate and describe the method of representing evolution.

We assume that if there are M individuals currently alive ($M \leq N$) then the predator encounters prey at rate λM, for some constant λ, and so the time taken to find a prey item is drawn from an exponential distribution with this rate. The actual individual that the predator encounters is chosen at random from the M individuals currently alive. Recall that the predator must invest a time D in attacking this individual (i.e. D is effectively the handling time of the individual).

If at the end of a generation there are M individuals surviving, then these will be the parents of the N individuals that will begin the next generation. For each of these N individuals a parent is selected randomly (with replacement). The probability that a particular individual is selected is weighted by its fecundity (which is a decreasing function of investment in defence). Specifically, the probability that individual i is chosen to be the parent of a particular offspring is P_i where

$$P_i = \frac{\exp(-cD_i)}{\sum_{j=1}^{M} \exp(-cD_j)}. \qquad \text{eqn 21}$$

In simulations where defence is continuous, we assume that offspring take their parent's level of defence subject to a small perturbation drawn from a uniform distribution [$-\varepsilon$, ε]. If defence is discrete, the offspring take their parent's level of defence with probability ($1 - \mu$), the level one less with probability (μ /2) and the level one greater with probability (μ /2). In both cases, we have a reflecting boundary at zero, to prevent negative values of defence.

Unless otherwise stated, the following parameter values are used: a generation time T of 200, a maximum population size N_1 of 200, and an encounter rate λ of 0·01. This means that when the population is at its maximum, the expected time between prey encounters is $(\lambda N_1)^{-1}$, which equals 0·5 or 0·25% of the prey's generation time (T). The survival constant s is 0·12, so that while a D_i value of zero means that an attacked prey never evades capture, a D_i value of 10 gives a 70% chance of escape, and a D_i value of 20 gives a 91% chance. The cost of defence parameter (c) is set at 0·05. This means that an individual with $D_i = 10$ has only 60% the fecundity of an individual with no investment in defence, dropping to 37% for $D_i = 20$. The mutation parameters are set at $\varepsilon = 0{\cdot}05$ or $\mu = 0{\cdot}01$. Simulations start with $D_i = 0$ for all individuals.

For continuous variation in defence, Fig. 1, shows that the population evolves through time, settling down (after around 30 000 generations) to a situation where all individuals in the population have very similar investment in defence (close to 2 for the parameter values used in Fig. 1). There are small fluctuations in the population over time caused by the inherent stochasticity of the model, but overall the behaviour is very similar to that predicted by the theory of the last section: a single pure ESS. Hence, the key prediction of the theory appears to be maintained even though the number of attacks (A) is now a substantial fraction of N_1. Further if we take the average value of A in the last 10 000 generations of Fig. 1 (75·1) and substitute this into equation 11 we get an estimate for the ESS level of defence of 2·02. For comparison, the average value of D across the final 1000 generations in the simulation is 1·97. Quantitatively similar results have been obtained for other combinations of parameter values. Hence we conclude that the predictions of the theory for continuous variation presented in the last section are robust against violation of its assumption of low predation pressure.

We next turn to simulation of discrete defences. Figure 2(a) presents the equilibrium distribution of defensive investment across the population for the case where all defence levels in the set (0, 0·1, 0·2, … , 5·0) can be selected. The equilibrium distribution is similar to the continuous case shown in Fig. 1, in that there is a narrow spread of different levels, centred on 2·1 but ranging from 1·9 to 2·4. This spread is an inevitable consequence of the mutation mechanism, but qualitatively we see that the predictions of the model for a fine-grained discrete defence are very similar to the predictions for a continuous defence: exactly as indicated by the theory of the last section. Returning briefly to the analytic model of the last section, where again A/N is small, we can approximate $A(D)$ by

$$A(D) \approx \frac{N\lambda T}{N\lambda D + 1}. \qquad \text{eqn 22}$$

If we use this approximation in the analysis of the last section, it is easy to show that for the population simulated to obtain Fig. 1 we obtain the analytic prediction that the pure ESS strategy should be $D^* = 2{\cdot}13$. This is reasonably close to the value obtained by

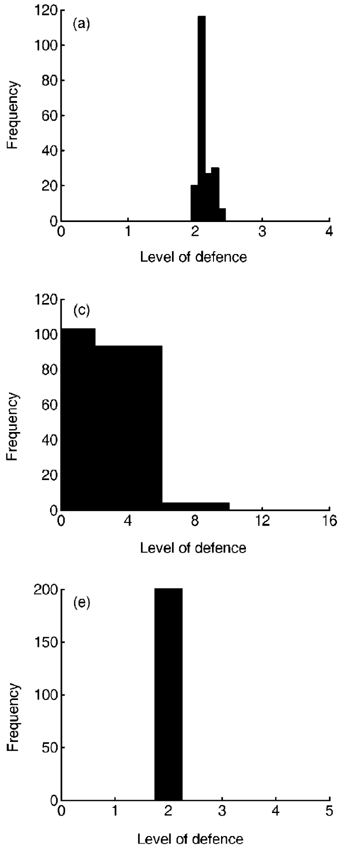

Fig. 2. The distribution of defence levels (*D*-values) adopted by individuals at the start of generation 30 000. All parameter values are as in Fig. 1, but now defence can only take a discrete number of values; (a) 0, 0·1, 0·2, … , 5·0; (b) 0·5, 1·5, … , 9·5; (c) 0, 4, 8, … , 24; (d) 0, 0·1, 0·2, … , 1·0 and (e) 0, 1, 2, … , 9. Parameter values: $T = 200$, $N_1 = 200$, $\lambda = 0{\cdot}01$, $s = 0{\cdot}12$, $c = 0{\cdot}05$, $\mu = 0{\cdot}01$.

the simulation. Similarly, for discrete level case of Fig. 2(a), we obtain the analytic prediction of a mixed ESS with individuals taking values 2·1 and 2·2, which again is close to the simulation results.

Figure 2(b) shows a representative distribution of defences for a simulation identical to that of Fig. 2(a) except that the defence is now more course grained, with allowable values [0·5, 1·5, 2·5, … , 9·5]. A similar pattern to that shown in Fig. 2(a) can be seen, with the two defence levels (1·5 and 2·5) either side of the ESS value predicted by the continuous case (~2·0) being most populous. Again, this is exactly as predicted by the analysis of the last section, where (using the approximation of equation 22) we predict a mixture of 1·5 and 2·5 is the ESS solution. In Fig. 2(c) the allowable defence levels are now even more course grained, being [0, 4, 8, … , 24], and the same trend is apparent with 0 and 4 being by far the most populous levels, which again coincides with the theoretical results.

The analytic solution of the last selection suggests that the type of polymorphism displayed in Fig. 2(c) is not inevitable for discrete defences and monomorphism is possible. An example of this is shown in Fig. 2(d), where only defence levels [0, 0·1, 0·2, … , 1·0] are allowed, all of which are below the ESS of the equivalent continuous model. As predicted, the ESS for this case is for all individuals to adopt the highest defence level possible (1·0), with a small number of individuals having values just below this, purely because of the mutation mechanism generating small levels of heterogeneity. It is also possible to find such a pure solution that is not one of the extreme solutions. Figure 2(e) shows the equivalent ESS for the situation where defence levels [0,1,2, … , 9] are allowed. In this case the analytic prediction is a pure solution $D = 2$, in complete agreement with the simulation results shown in the figure.

Hence, the conclusions of our analysis under the restriction that A is much less than N all seem to hold qualitatively in simulations where this restriction is relaxed. Further, quantitative predictions provided by the analysis provide a good approximation to those of the simulations.

Discussion

In this section we relate the finding of our models to our three stated aims.

Aim 1: To explore the generality of the model prediction of Leimar et al. *(1986) that the predicted ESS of defence should be expected to be a pure strategy in models that represent defence as a continuous trait*

We present a model of a continuously varying defence that is solved explicitly for evolutionarily stable strategies. We are able to demonstrate analytically that this simple but quite general model always has a unique ESS, which is always pure. This strategy may involve all members of the population adopting no defence, or all members of the population making the same non-zero investment in defence. We are able to provide a quantitative expression delineating these two regimes. Non-zero investment in defence is more likely when predation pressure on the population is strong. Increases in predation pressure can be seen to cause increases in the ESS for level of defence. Our general analytical solutions are obtained in the limiting case where predation pressure is not very high, but our simulations demonstrate that the qualitative conclusions also hold when predation pressure is very high. Further, the quantitative expression for the ESS level of defence derived analytically for the limiting case of low predation pressure still provides a good approximation when this condition does not pertain.

Aim 2: To search for general rules about the nature of the mixed ESSs that have previously been demonstrated for situations where levels of defence are discrete

Again our results were obtained by complete ESS analysis of a simple general model in the limited case where predation pressure is low, combined with simulations that demonstrate the generality of the analytical conclusions without any restriction on level of predation pressure. In common with Till-Bottraud & Gouyon (1992) and Speed *et al.* (2005) we find that models with discrete descriptions of defence can predict mixed ESSs with stable within-population variation in investment in defence. Further, unlike previous studies we can definitively say that this behaviour is due to the nature of the defensive trait. This assertion follows since our models that produce only pure ESSs and those that produce mixed ones are identical in all respects other than in their description of the levels that the defensive trait can take. Further still, we demonstrate that a mixed ESS is not the inevitable outcome of a discretely expressed defence, since both our analysis and simulation models demonstrate that the unique ESS can be either pure or mixed. The pure ESS can have non-zero levels of defensive investment.

Further, for our simple but general model, we can make clear statements about the nature of the mixed ESSs, specifically that they have the following characteristics.

1. The mixed ESS can be a combination of no more than two defence levels.
2. The two levels in a mixed ESS must be nearest neighbour levels in an ordered list of the levels that the defence can take.

This has important ramifications for judging the importance of mixed ESS explanations for the prevalence of automimicry in the natural world. It suggests that mixed ESSs may be an explanation where defence is discrete and where individuals can be divided into two categories (for example with toxins either fully expressed or not expressed at all), and where we are confident that no intermediate form of defence (partially expressed toxins) could exist. If these criteria cannot be satisfied, then explanations for automimicry are likely to lie in phenomena other than mixed ESSs. Such causes may obviously be external to the prey such as variation in available foods that confer toxicity (e.g. Brower, Edmunds & Moffitt 1975; Brower *et al.* 1984). Furthermore if sequestration and biosynthesis of toxins is costly then variation in the resource richness of microhabitats within which the prey exists may cause intrapopulation variation in investment in chemical defence. In cases of defensive sequestration, such as reflex bleeding in ladybirds (Holloway *et al.* 1991), the defensive act often causes depletion of a defensive resource: again small-scale geographical variation in predator threat could lead to variation in defence within a breeding population. In addition causes of variation may have some internal heritable component (see Eggenberger & Rowell-Rahier 1992; Muller *et al.* 2003).

Aim 3: To understand how the pure and mixed strategies predicted by the two types of defences can be reconciled with practical and philosophical difficulties in defining a given defence unambiguously into one of these two categories

Another important conclusion from our work is that our models give substantially similar solutions in the case where the defensive trait is continuous and in the case where the trait is discrete but fine grained (cf. Figs 1 and 2a). Although the discrete case may formally be a mixed ESS, this ESS will involve only two levels and these levels will be nearest neighbours in an ordered list of allowable levels. This, in turn, means that the mixed ESS will be practically identical to a pure ESS if the discrete defence is fine grained. Further, we demonstrate that the two levels of the mixed ESS in the discrete case, will straddle the pure ESS level from the equivalent model with a continuous defence. Hence, the apparent contradiction between existing models with continuous and discrete defences can in fact be practically reconciled: whether a defence is continuous or discrete with a fine-grained range of available levels makes no practical difference to the extent of variation in defence that we should expect to measure across a population. Only when there is measurable difference between nearest-neighbour levels of the discrete defence can we expect to find mixed ESS behaviour that is practically different from a pure ESS.

Conclusions

Whereas aposematism and mimicry are well-studied components of prey defences, the evolutionary stability of constitutive secondary defences has received surprisingly little attention. Though some authors separately suggest that pure (Leimar *et al.* 1986) and mixed ESS states (Guilford 1988, 1994) for secondary defences may be evolutionarily stable, there has been no general analytical demonstration of the conditions that define these states. Yet the distinction between mixed and pure ESS states is important, especially since some authors have argued that automimicry (in which some proportion of a population are defended, while another proportion are undefended) may arise as a mixed ESS state (Guilford 1994) and subsequently others have carried out detailed chemical analyses in order to see whether mixed ESS states for chemical defence can be observed in nature (Holloway *et al.* 1991). In fact Holloway *et al.* found that chemical defences in the Seven Spot Ladybird were (i) continuous in form and (ii) did not manifest automimicry.

We have shown that there is likely to be a single, pure ESS state when defences are continuous in nature (although it is possible that there is more than one such pure ESS). A unique mixed ESS solution can occur when two defended forms exist in a population and straddle the value for the notional pure ESS from the continuous model, although it is also possible that one of these two forms will be the unique pure ESS. When discretely varying defended forms do not straddle the pure ESS value, then a pure solution is predicted, with the defensive form nearest to this pure value being monomorphic (again if there is more than one continuous pure ESS, there is likely to be such an ESS in the discrete model corresponding to each such ESS). Furthermore, our capacity to detect mixed ESS states is limited by the degree to which adjacent discontinuous defences are differentiated; as differentiation between discreet states decreases, so phenotypic variation in defence caused by mixed ESS states reduces. In natural systems our mixed ESS solution will converge on the pure state as the difference between defended forms becomes too small to measure.

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