

A user guide to FDS in the wild: the construction and diagnosis of payoff matrices

Supplementary material for figure 2 is divided into: i) payoff matrix construction for natural populations, ii) tenary plots and cyclical dynamics, iii) non-linearity in the payoff matrix and correlational selection gradients, and iv) emergent properties of genotypic ESS analysis.

i) Payoff matrix construction for natural populations

The payoff matrix is useful in diagnosing the kind of mating system dynamics that is exhibited by a given social system (e.g. lizards: true RPS, damselflies: pure apostatic RPS, isopods: true and apostatic RPS, tristily: Fisherian sex-ratio apostasis). ESS analysis treats pair-wise invasion conditions of morphs, even in cases with 3 or more types. A payoff matrix is analogous to a population genetic analysis of allele invasion, if payoffs are expressed as the additive effects of alternative alleles when infinitesimally rare. However, payoffs in nature are best expressed in terms of ecological expressions for rarity, which can be gleaned from field data (e.g. territorial interactions, Sinervo 2001ab). Our ESS analyses of the four mating systems in figure 2, do not express payoffs as additive genetic effects, rather the payoffs are expressed for phenotypes. The genotypes are listed under each phenotypic strategy. In principle, we could have estimated the additive genetic effects, if we had access to primary, rather than published data. For the lizard RPS mating system (Sinervo and Clobert 2003), there is an additive effect of b alleles on male cooperation, relative to y alleles, since y has a neutral effect on cooperation. In the isopod mating system, additive genetic effects are implicit since rare homozygote combinations do not obscure payoffs of alleles that govern alternative strategies. For example, in isopods the population-wide frequency of β and γ alleles are so low that frequency of homozygote types, $f(\beta\beta)$ and $f(\gamma\gamma)$, can be ignored [Shuster and Wade 1991a]). Thus, for the isopods, payoffs reflect the additive genetic effects of α , β and γ alleles invading a largely α -genotypic background. As such the values in the payoff matrix reflect the additive genetic frequency-dependent effects.

Estimating equations for relative fitness, w_i , the first step in constructing the elements of a fitness payoff matrix, the $\omega_{i,j}$, which typically assume linear relations for fitness and frequency:

$$w_i = a_0 + \sum a_{i,j} \times f_{i,j} \quad (1),$$

where w_i is relative fitness of the i th type, a_0 is a constant, and the $a_{i,j}$ estimate social selection gradients [SSG] describing FDS of the j th type on the i th type, $f_{i,j}$. We describe payoff matrix construction for two cases of frequency, $f_{i,j}$, in the four mating systems: absolute numbers of neighbors (f_{i,N_j}) for isopods and lizards, and a population-wide relative frequency ($f_{i,j}$) for damselflies and tristylous flowers. Below, we also describe how non-linearity in the payoff matrix arises from correlational selection [CS] on the traits of interactors. Non-linearity and CS may arise from FDS among types (3-way interactions among diagonal elements and off-diagonal elements). Non-linearity can also arise because of CS on traits affecting self-attraction or self-repulsion (i.e. within diagonal elements). We initially assume linearity (Eqn. 1) and then relax this assumption by computing cross-product (*c.f.* CSG) relations for FDS in all 4 mating systems.

Computation of SSG using ANCOVA, and conversion of SSG to payoff matrices is described in Sinervo (2001ab), Sinervo and Lively (1996), and Sinervo et al. (2000) for social systems with coarse neighborhoods and few neighbors (e.g. territorial lizards or isopods residing inside spongocoels). Each column of the payoff matrix (or cluster of 3 histograms in figure 2), $\omega_{i,j}$, reflects a neighborhood where each type enters into an interaction with a single common

type. The number of neighbors ($N_{i,j}$) is also a behavioral trait that varies among types owing to genetically determined settlement behaviors expressed by males (Sinervo and Lively 1996, Zamudio and Sinervo 2001a, Sinervo et al. 2006b) or behaviors expressed by females (female copying: Shuster and Wade 1991, density-dependent competition: Sinervo et al. 2001, or self-recognition female mate preferences: Sinervo et al. 2006b). Fitness of rare and common types, the elements of the payoff matrix (represented as histograms, fig. 2), the $\omega_{i,j}$, are derived from SSG by computing w_{rare} and w_{common} (i.e. $\omega_{i \neq j} = w_{rare}$ in the upper and lower off-diagonal elements, $\omega_{i=j} = w_{common}$ in the diagonal elements). In figure 2 we standardized the payoff matrix by dividing $\omega_{i \neq j}$ by $\omega_{i=j}$. This sets the relative fitness of w_{common} in a neighborhood of common types, the diagonal elements, to a relative fitness of 1. Common types experience only common neighbors at an absolute rate $f_{i,N_i} = N_{i,i}$. In contrast, both rare types, k and h , are surrounded by the common type i ($f_{k,N_i} = N_{k,i}$, $f_{h,N_i} = N_{h,i}$). Values for frequency of each type when rare (f_{k,N_i} , f_{h,N_i}) are substituted into equation 1 along with empirical estimates of the $a_{i,j}$ to yield one column of the payoff matrix (e.g., cluster of 3 histograms). Carrying out similar calculations for each type as common yields all 3 columns of the payoff matrix (or 3 different clusters of 3 histograms).

In all mating systems, ANCOVA was used estimate the $a_{i,j}$ as partial regression coefficients. This allows one to consider pair-wise cases in which one type is ignored in a given interaction (i.e. $f_{k,N_i} = 0$) that considers frequency of a rare type h surrounded by i ($f_{h,N_i} = N_{h,i}$) or common type i surrounded by i ($f_{i,N_i} = N_{i,i}$). The converse rare type k is also considered in another off-diagonal element (i.e. $f_{h,N_i} = N_{h,i}$) in a common world of i (i.e. $f_{i,N_i} = N_{i,i}$), while type h is ignored ($f_{h,N_i} = 0$) in that particular pair-wise interaction. Below, we relax this assumption and allow for three way interactions, rather than just the simple pair-wise interactions.

The side-blotched lizard RPS mating system. The payoff matrix for side blotched lizards, *Uta stansburiana*, was derived from paternity data. See Sinervo (2001a) for a detailed description. However, other equations for *bb* cooperation and the RPS are found in Sinervo et al. (2006b).

The RPP mating system of isopod male morphs. The payoff matrix for the isopod, *Paracerceis sculpta*, was computed from mating rules for paternity success (*Paternity_{success}*, table 1: Shuster and Wade 1991a). We also used data on frequency distribution for number of females [$N_f(f_{N_\alpha}$, f_{N_β} , f_{N_γ})] settling in spongocoel neighborhoods as a function of morph frequency (f_{N_α} , f_{N_β} , f_{N_γ} , Table 2: Shuster and Wade 1991a) to compute fitness payoffs, which are similar to payoffs in figure 2. We describe the isopod example in detail as this case parallels the ESS analyses of the mating systems of lizards, damselflies and tristyls. Fitness of male isopods is given by:

$$w_i = \text{Paternity}_{\text{success}}(f_{N_\alpha}, f_{N_\beta}, f_{N_\gamma}) \times N_{\text{females}}(f_{N_\alpha}, f_{N_\beta}, f_{N_\gamma}) \quad (2),$$

The mating rules of Shuster and Wade (1991a) assume linear relations of morph frequency on fitness. Our payoff matrix for isopods (figure 2) reflects this assumption. Here, we describe approximations of non-linear FDS for isopods (e.g., arising from female copying behavior, which is describe for w_α , in the correlational selection surface in the rightmost column of figure 2) Notice that w_i in equation 2 is due to sequential episodes of selection. If female settlement depends on f_{N_α} , this will influence fitness directly or indirectly via paternity success. Indirect effects on paternity success are likely if β and γ males are attracted to spongocoels with many females or α males. If any one condition is met, male fitness will take on a more complex form involving 3-way interactions, because paternity success is not independent of female settlement, which has cascading effects on f_{N_α} , f_{N_β} and f_{N_γ} . Females do appear to settle as a function of f_{N_α}

and N_f (Shuster and Wade 1991b) and therefore, this can alter behavior of $f_{N\beta}$ and $f_{N\gamma}$. Equation 2 should be revised to accommodate this behavioral complexity in the social system dynamic:

$$w_i = \text{Paternity}_{\text{success}} [f_{N\alpha}, f_{N\beta}, f_{N\gamma} | N_f(f_{N\alpha}, f_{N\beta}, f_{N\gamma})] \quad (3).$$

The conditional relationship for paternity fitness of each morph (equation 3), given settlement of females (and males), regardless of the exact functional form, can be empirically approximated with a series of terms such as f_{α}^2 and $f_{\alpha} \times N_f$. Equation 4 is analogous to a Taylor series expansion commonly used to approximate non-linear functions. In equation 4, the terms with expanding exponent after the term $b_{N\alpha, N\alpha} \times f_{N\alpha}^2$ take the form $b \times f_{N\alpha} \times f_{N\beta} + \dots + c \times f_{N\alpha} \times f_{N\alpha} \times f_{N\beta} + \dots$

$$w = a_0 + a_{\alpha} \times f_{N\alpha} + a_{\beta} \times f_{N\beta} + a_{\gamma} \times f_{N\gamma} + b_{\alpha, N_f} \times f_{N\alpha} \times N_f + b_{N\beta, N_f} \times f_{N\beta} \times N_f + b_{N\gamma, N_f} \times f_{N\gamma} \times N_f + b_{N\alpha, N\alpha} \times f_{N\alpha}^2 + \dots \quad (4),$$

The $b_{i,j}$ are cross products for fitness effects of two interactors for $i \neq j$, similar to a CSG (but not standardized like the CSG), or quadratic effects of selection for $i=j$. (N.B., the first subscript, i , should be included in each term of Equation 4 but for clarity it has been omitted). Standardizing fitness (i.e. Lande and Arnold 1983) is unnecessary at this point, since fitness standardization is ultimately achieved when the w_i are converted to the elements of the payoff matrix, the $\omega_{i,j}$ (see methods above). Nevertheless, one could convert equation 4 to a form in which relative fitness (dependent variable) and independent variables are standardized (Lande and Arnold 1984) and thus the $b_{i,j}$ would reflect quadratic selection gradients and correlational selection gradients of a typical fitness analysis. Notice that quadratic terms < 0 often have a biological interpretation of self-limiting (concave down), if the fitness surface includes a maxima. For example, in the equation for w_{α} the significant quadratic parameter estimated for SSG $b_{\alpha, \alpha}$ as a function of $f_{N\alpha}^2$ reflects the fact that α males are despotic and self-limiting. Coefficients for quadratic terms > 0 have a biological interpretation of social facilitation (concave up), if the fitness surface is monotonic and increasing. Even terms in the series expansion such as $c \times f_{N\alpha} \times f_{N\alpha} \times f_{N\beta} = c \times f_{N\alpha}^2 \times f_{N\beta}$ have an interesting biological interpretation, if statistically significant. Such a term would imply that the fitness gains of β - males are dependent on a quadratic (despotic) effect of α - males.

In preliminary models, we included quadratic terms for $f_{N\beta}^2$ and $f_{N\gamma}^2$ and terms for $f_{N\beta} \times N_f$ and $f_{N\gamma} \times N_f$, however, these quadratic terms for β - and γ - were not significant in the final model. The final ANCOVA model of SSG indicates that at least one $a_{i,j}$ coefficient for $f_{N\alpha}, f_{N\alpha}^2, f_{N\beta}$ or $f_{N\gamma}$ was significant in at least one of three w_i equations for at least one male morph (i.e. $i=\alpha, \beta, \gamma$). Moreover, the fitness surface for α - males, w_{α} , also included a significant CSG for $f_{N\alpha} \times N_f$. The three empirically estimated non-linear equations for FDS, w_i , were used to derive the elements of the payoff matrix, $\omega_{i,j}$, for the isopod mating system (assuming spongocoel neighborhoods of a given average size for each male morph, computed from Table 2 Shuster and Wade 1991a).

Significant linear coefficients for the $a_{i,j}$ SSG of $f_{N\beta}$ and $f_{N\gamma}$ suggests that w losses of α - males (and w gains by β - and γ -) are captured by the assumption of linearity (in the absence of any significant quadratic terms). The linear effects of $f_{N\beta}$ and $f_{N\gamma}$, the number of β - and γ - males in spongocoels, may arise because of additive frequency-dependent genetic effects of these alleles (as noted above). Alternatively, the population-wide frequency of the homozygote classes $\beta\beta$ and $\gamma\gamma$ may be so rare (i.e. $f(\beta\beta) \approx 0, f(\gamma\gamma) \approx 0$) that there is no statistical power to leverage any effects of curvature associated with these strategies. In contrast, the number of α - males in

spongocoel neighborhoods, $f_{N\alpha}$, is always at high frequency. Thus, population-wide frequency of α - males, $f(\alpha)$, will increase to a high enough frequency to affect fitness of w_α . The quadratic effects of α - in spongocoel neighborhoods, which we detected in the dataset of Shuster and Wade (1991a), arise from non-linear frequency effects ($f_{N\alpha}^2$) of α - male neighbors. Experiments in which the $f_{N\alpha}$, $f_{N\beta}$, and $f_{N\gamma}$ are varied, would be necessary to determine causation. The paternity rules developed by Shuster (1989) reflect such experiments for α - common. In contrast, estimating the invasion conditions of α - males into populations with a high frequency of β - and γ - may be suspect since they are never common. We assumed that β - and γ - could never get to a high frequency (e.g., we interpolated the linear mating rules of Shuster and Wade 1991a). This is reasonable if females avoid settling in spongocoels that only contain β - and γ - males, which is indeed consistent with data in Table 2 of Shuster and Wade (1991a) in which spongocoels that only contain β - and γ - males often lack females. Thus, female copying (Shuster and Wade 1991b) may be driven by a female preference for spongocoels with α - males or by an aversion to cells lacking α - males.

Regardless of which is the case, patterns are consistent with the hypothesis that isopod females settle based on some α - male cue (or vice versa). In the next phase of sexual selection, despotic α - males compete over spongocoels containing many females. At high frequency, α - males become self-limiting. The β - and γ - males settle as a function of these two antecedent phases of sexual selection, thereby reducing the fitness of α - males. We refer to the isopod mating system as RPS apostasis since the α - male strategy is despotic (i.e. analogous to the despotic Rock = O strategy of lizards). Even though both β and γ strategies are cryptic (i.e. analogous to the Paper = Y strategy of lizards), the three morphs still exhibit intransitive RPS relations (equation 2, in the text). Thus, the isopod RPS, which lacks an explicit S strategy but contains two distinct cryptic P strategies, can be considered a degenerate form of the RPS mating system. The mating system is apostatic because both β and γ beat common α , thereby exhibiting apostatic selection. The trimorphic apostatic RPS mating system may be common in fish such as blue-gilled sunfish and salmon (Gross 1984, 1985, 1991). In many fish mating systems, a parental (i.e. care-giving) but despotic strategy is required for invasion of sneaker males. In such mating systems, sneaker tactics can never rise above some critical frequency that is largely dictated by the dynamics among despotic parental males, and female preference for these males that care for their young.

The apostatic RPS mating system of damselflies. In populations of diffusely interacting types such as female damselfly morphs of *Ischnura elegans*, the payoff matrix was constructed from a relative frequency (i.e. proportion) that was arbitrarily defined to be rare. In figure 2, we chose $f_{\text{rare}} = 0.05$ to compute the $\omega_{i,j}$ of the payoff matrix. Setting $f_{\text{rare}} = 0.05$ has utility in that it can serve as a statistical criterion for assessing if one morph is absent in a population (with $P < 0.05$). In principle, one could use other biologically relevant criteria of rarity such as rare alleles in the context of gene flow (Slatkin 1985) or alternative types that affect probability of dispersal in a metapopulation (Sinervo et al. 2006b), which is intimately related to the spatial scale of social selection (Svensson and Sinervo 2004). To compute payoffs, we set the *ith* rare type, $f_i = 0.05$ and *kth* common type to $f_k = 0.95$. The *lth* type is assumed to be absent from the given pair under consideration ($f_l = 0.00$), but it affects fitness in pairs where it is either rare or common. This procedure only differs from coarse neighborhoods (lizards and isopods) in that population-wide frequency substitutes for absolute frequency that governs FDS in coarse neighborhoods ($N_{i,j}$).

Fitness equations for damselflies, $w_i = \sum a_{i,j} \times f_{i,j}$, were presented in Svensson et al. (2005). We computed payoffs of rare and common types in a pair-wise fashion from their equations. Below we show that the damselfly mating system also satisfies conditions for an apostatic RPS.

Tristyly mating systems. The diffuse spatial scale of FDS for tristyly is similar to that observed in damselflies. Movement of insect pollinators within an isolated population generates the FDS of tristyly. To derive a payoff matrix for tristyly in *Lythrum salicaria*, we first computed $W_i = a_0 + \sum a_{i,j} \times f_{i,j}$ using repeated measures ANCOVA for seed set (repeat = W_L, W_M, W_S , W is absolute seed set, data derived from figure 2 in Ågren and Ericson 1995) as a function of the frequency of each pair of rare and common types in the populations. Corresponding data on morph frequency in each population were derived from table 1 of Ågren and Ericson (1995). ANCOVA of rare and common types cannot include all 3 frequencies as independent variables since such a regression would be singular, given that $f_L + f_M + f_S = 1$. Accordingly, repeated measures ANCOVA were carried out using frequency pairs for a given computation of rare and common types (3 regressions in total). All 3 regressions of seed set were significant for the 3 pair-wise cases of dependent variables of morph frequency (i.e. f_L and f_S ; f_L and f_M ; f_S and f_M as covariate pairs in 3 separate ANCOVAs) ($P < 0.05$). Thus, differences in fitness of tristyly morphs in the payoff matrix, when comparing rare types within a class of common types, are statistically significant. We arbitrarily set $f_{rare} = 0.05$ and $f_{common} = 0.95$ in the payoff matrix for tristyly to compute the $w_{i,j}$.

Fitness estimates for *L. salicaria* based on seed set also account for fitness via pollen success. The pollen, which enhances seed set of a given rare style morph, must be derived from the two other floral types (this is equivalent to the Fisherian consistency requirement of sexual parents, Kokko et al. [2006]). Effects of FDS on seed set arise because pollen is limiting in these populations (Ågren and Ericson 1995). Thus, a rare morph advantage in seed set is indicative of an overabundance of pollen coming in from a compatible anther type. However, one cannot determine which of the two anther types pollinated the seed set of a given rare style type.

ii) Tenary plots, cyclical dynamics, and the categorization of mating systems

The RPS payoff matrix of lizards predicts cyclical dynamics (Sinervo 2001a). Moreover, the payoff matrices for other mating systems like damselfly morphs also predict cyclical dynamics. Cycling dynamics of the damselflies are generated because of a subtle fitness intransitivity similar to an RPS payoff matrix. Intransitive fitness relations in the payoff matrix will generate cycles, even in the case of pure apostasis. RPS Cycles will occur for female damselflies ($A \rightarrow IO \rightarrow I \rightarrow A$; figure 2) because:

$$W_{IO,rare} > W_{I,rare} > W_{A,common} \quad (5)$$

$$W_{A,rare} > W_{IO,rare} > W_{I,common} \quad (6)$$

$$W_{I,rare} > W_{A,rare} > W_{IO,common} \quad (7).$$

We suggest that this kind of mating systems should be termed an apostatic RPS. An apostatic RPS differs from a true RPS in that one rare strategy loses to the common strategy in the true RPS, whereas in an apostatic RPS the common type loses to both rare types. Notice that RPS payoff matrix of lizards has 1 set of interactions that is an RPS apostasis ($W_{common Y}$), but the other 2 sets are true RPS interactions (i.e. $W_{common O}$ and $W_{common B}$). The true RPS and apostatic RPS mating systems will both generate cycles of FDS, thus CS that affects payoffs may become self-reinforcing (this point is explained in greater detail, below, or in Sinervo 2001).

The cycling dynamics of lizards are actually more complex if one considers both male RPS frequency cycles and density-dependent cycles driven by female morphs (Sinervo 2001b,

Alonzo and Sinervo 2001). The two attractors of lizards (from Alonzo and Sinervo 2001) result from RPS cycles (counterclockwise on the ternary plot: $B \rightarrow O \rightarrow Y \rightarrow B$) and the destabilizing effect of the female-morph density cycle (2-yr cycle), which accelerates the male RPS to high O frequency every 4 years (Sinervo 2001b). The mate choice model of Alonzo and Sinervo (2001) is based on FDS of the female density cycle (r-K strategy, Sinervo et al. 2000), FDS of male RPS cycles (Sinervo and Lively 1996), FDS generated by Fisherian sex-ratio, and hypothesized context dependent mate choice, which gives an advantage to rare female morphs that mate with rare male morphs, thereby producing rare morph progeny during RPS and r-K cycles.

The isopod mating system may have RPS cycles ($\alpha \rightarrow \beta \rightarrow \gamma \rightarrow \alpha$) since it exhibits fitness intransitivity with elements of a true RPS (equation 9) as well as RPS apostasis (equations 8, 10):

$$W_{\beta,rare} > W_{\gamma,rare} > W_{\alpha,common} \quad (8)$$

$$W_{\gamma,rare} > W_{\beta,common} > W_{\alpha,rare} \quad (9)$$

$$W_{\alpha,rare} > W_{\beta,rare} > W_{\gamma,common} \quad (10).$$

The cycling dynamics of isopods are also complex as this mating system involves FDS of a similar sort in the guise of a sex transformer (*Tfr*) locus (Shuster and Sassaman 1997). The destabilizing effect of the *Tfr* locus perturbs the population from the Fisherian sex ratio in a cyclical fashion as each morph of the *Ams* locus invades in turn (α, β, γ). Thus, the scatter of points shown for isopods may be best represented by cyclical dynamics (see figure 4 in Shuster 1989). The two attractors reflect the computed attractor from the payoff matrix (far right) and a hypothesized attractor given stability generated by Fisherian sex ratio (far left). Conversion of females to α males (as rare *Tfr* allelomorphs 1 vs. 2 invade in turn) increases frequency of α males, allowing invasion of β and γ males. While a formal theoretical treatment of the isopod mating system is beyond the scope of our review, such theory should integrate epistasis between the *Ams* and *Tfr* loci and how this epistasis perturbs Fisherian sex ratio (*c.f.* the lizard RPS).

Cycles are non-existent in tristily because FDS in simultaneous hermaphrodites will generate very strong stability for equal (1/3) morph frequency (Eckert and Barrett 1992, 1995, Lloyd and Webb 1992ab). FDS is thus due to Fisherian sex ratio stabilization of rare pollen types relative to alternative common stigma types. Nevertheless, random sampling in small populations will constantly perturb the system from the attractor (Eckert and Barrett 1992, 1995) but FDS will generally return rare types back to equilibrium, unless one happens to be lost due to genetic drift or during colonization events (Eckert and Barrett 1992). During the return to the attractor and at this equilibrium, powerful CS among traits constantly promotes self-reinforcing genetic correlations that shape and refine each floral type over evolutionary time (see below).

iii) Non-linearity in the payoff matrix and correlational selection gradients

A fundamental thesis of our review holds that correlational selection is not just a property of individual-based traits, but that CS is a property of FDS between interactors [such as signaler-receivers in male-male competition (lizards and isopods), antagonistic FDS between the sexes (damsselflies) or between males (anther) and females (style)]. The only inter-individual CSG on traits that has been computed in the primary literature is for the RPS mating system of side-blotched lizards. In this case, settling *bb* males benefit from attraction to self-color (e.g. blue color), but such a benefit arises from an even deeper self-recognition of genetic similarity (Sinervo et al. 2006b). This reflects a mutual attraction of two males that share genetic factors for self-recognition and self-attraction during male settlement. The cooperative relationship of dyadic *bb* males is enhanced by powerful correlational selection that couples self-recognition to

the OBY locus, and to other traits that stabilize cooperation. Thus, significant CS on *bb* male fitness as a function of the number of *b* alleles carried by the male and the number of *bb* males in the male's neighborhood (fig. 2, top row, right panel) is diagnostic of positive FDS on *bb* dyads.

The genetic factors under CS have been mapped in side-blotched lizards. The traits include the OBY locus and at least 3 unlinked loci for self-recognition (of genetic similarity, Sinervo et al. 2006b). In the other three mating systems of figure 2, the specific traits under CS between the interactors have not been identified. However, we estimated CSG that are due to FDS among morphs in each of these three mating systems. Significant CS among the frequency combinations of types strongly implies that specific traits are under CS in each mating system including, but not limited to: i) female copying behaviors in isopods, ii) learning mechanisms and perceptual biases in male damselflies for female morphology, iii) relative style and anther height in tristylous morphs and other physiologically-based incompatibility systems, which reinforce morphological incompatibility, thereby promoting more efficient blocks to selfing.

CS, which is expected in all social systems, can generate 3-way interactions in the payoff matrix (eqn 4) that can be approximated by computing synergistic fitness effects among morphs. The expansion of terms in equation 4 for the isopod mating system provides a statistical method for testing for CS and fitting a parametric fitness surface. This method is only a substitute for an analysis of the specific traits or loci that generate the CS of FDS (e.g., number of *b* alleles in male \times number of *b* alleles in cooperating neighbors, figure 2; Sinervo et al. 2006b).

In isopods, fitness gains of α - males depend on harem size. Paradoxically modest-sized harems generate the highest α - male payoffs. β - and γ - males obtain high fitness through female mimicry (β -) or crypsis (γ -) as harems become larger (Shuster 1989). In addition, high $f_{N\alpha}$ leads to mating skew among α males. Many α males end up in spongocoels lacking females and get zero fitness. Notice from equation 4 that male fitness involves a term arising from the cross-product of $f_{\alpha} \times N_f$, which we treat as a CSG arising from α male settlement behavior and female (copying) settlement behavior (N_f). CSG for α -, β - and γ - male fitness may also involve genetic epistasis between two loci (Shuster and Sassasman 1997): the *Ams* governs proportions of each alternative male type and *Tfr* can perturb female harem size in the population via primary and operational sex ratio (Shuster 1989). In summary, fitness surfaces for the RPS apostatic mating system of isopods includes stabilizing selection on α males (e.g. despotic males), correlational selection on the genetic factors contributing to mating skew in harems, such as genetic epistasis between the *Tfr* locus, which converts females into males (and vice versa) as a function of *Ams*.

The surfaces in damselflies may be non-linear owing to asymmetries in the rapidity of male learning of female-morph search images (Fincke 2004). Only one surface (shown in Figure 2, right most panel for the damselfly mating system) exhibited significant CS: fecundity of I morphs is strongly enhanced by a high frequency of A and IO morphs. Thus, A and IO female morphs facilitate high fitness of the I female morph, a three-way interaction.

The fitness surfaces in tristylous mating systems may be non-linear perhaps owing to: i) asymmetries in pollen transfer by each anther type and ii) pollen tube competition on styles and iii) disruptive selection against common types. In support of case iii), we observed significant disruptive selection owing to intermediate frequency of f_M^2 on all three tristily forms, and also for f_S^2 on all tristily forms. In support of case i) and ii), we observed significant CSG due to an $f_M \times f_S$ interaction on the seed set of the L morphs, (see Figure 2, right panel, lower row for the pattern for L) and a significant but weaker effect on the S morph. An $f_M \times f_S$ CSG yields higher

seed sets for L when either M or S is rare (e.g. fitness peaks in corners), but not when M and S are common (e.g. fitness saddle). Notice that pollen will be more efficiently transferred to styles of the L type, relative to M or S styles, given that a long style more readily intercepts pollen from pollinators (Lloyd and Webb 1992ab). An $f_M \times f_S$ CSG (and fitness saddle) may have a simple biological interpretation. Given the hypothesized ability of pollinators to more efficiently transfer M or S pollen to L styles, pollen competition (from M and S plants) may be more intense on L styles, than on either M or S styles. Thus, a fitness valley in seed set of L styles, when M and S are both common, may arise from intense competition between M and S pollen, which results in pollen tube interference, thereby reducing seed set of L styles. Thus, pollen tube competition might limit the ability of pollen to reach the ovary on L style plants, but such pollen tube competition on short or medium style plants is not as intense. The origin of other physiological incompatibilities, such as other factors that limit selfing per se, occur via emergent properties among loci that govern the genetic basis of tristily. Deleterious recessives can build because of strong disassortative mating, which is always present in tristylous mating systems (genetics of tristylous mating systems are further discussed in section **iv**, below).

As noted above, epistatic interactions will build up during mating system evolution due to correlational selection within and between style-anther types. For example, even though tristily per se already promotes outcrossing (Darwin 1977), other as yet unidentified physiological factors limit selfing within floral forms (Lloyd and Webb 1992ab), thereby reinforcing the self-incompatibility due to floral morphology. Mating system evolution in tristily is expected to lead to CS that limits inbreeding via many other incompatibility systems besides the physical position of anthers and styles. Such CS is advantageous since it further enhances the morphological block to selfing. Selfing is considered to be a fundamental driver of tristily evolution (Lloyd and Webb 1992ab). Inbreeding and selfing are also frequency dependent processes driven by seed dispersal.

In summary, three-way interactions in tristily (e.g. $f_M \times f_S$ CSG on W_L) would have been missed if we had constructed a payoff matrix for tristily with the simple assumption of pair-wise interactions and linear relationships among players (e.g., histograms bar, ignoring the gradients and arrows). Similar three-way interactions are present between cooperating $B=bb$ genotypes or among B, O and Y male morphs of lizard RPS mating system [see Sinervo et al. 2006b], among females and α -, β - and γ - males of the isopod mating system (figure 2, right panel, 3rd row), or among I and common A and IO morphs of damselflies (figure 2, right panel, 2nd row).

iv) Emergent properties of genotypic ESS analysis

Most ESS models typically ignore genetics and focus on the interactions of phenotypes. Such simplification is possible because morph-based mating systems often express dominant alleles. Indeed, over evolutionary time there are good reasons why modifiers will evolve, under the action of FDS, to increase dominance of alleles affecting alternative strategies. Assume that a homozygous type is the most efficient at obtaining fitness from a FD interaction with another genotype. Given that heterozygotes are always more common than homozygotes, CS will refine invasion abilities of heterozygotes to closely match levels of expression seen in one homozygote. Thus, dominance of alleles should be common for the invasion of one or more strategies. Indeed, dominant alleles are present in all four mating systems that we considered.

Despite the simplifications of an ESS analysis of phenotypes that is afforded by dominant alleles, an over-reliance on results of phenotypic models is dangerous. The genetics of mating systems lead to emergent properties that are only explicable in terms of genotypic payoffs. The simplification afforded by phenotypic ESS analysis is, however, also pragmatic. Estimating the

payoffs for all combinations of genotypes may be impractical in nature (Sinervo 2001b). In lizards, a genotypic payoff matrix would require computation of a 36-element payoff matrix of genotypes compared to a simpler 9-element payoff matrix for phenotypes. It is for pragmatic reasons that the payoffs of FD interactions are often based on phenotypes (e.g., $O = oo, bo, yo$; $B = bb$, $Y = by, yy$). Data collected in nature rarely allows for the luxury of observing FDS on all genotypic combinations. The evolution of dominance in such situations (as noted above) will, however, ameliorate some of the limitations in inference built into phenotypic ESS analysis (Sinervo 2001a). Future empirical work should focus on estimating FDS arising from additive genetic effects (e.g., Sinervo and Clobert 2003) and epistatic genetic effects (exemplified by the isopod mating system [Shuster and Sassaman 1997] or cooperation among *bb* male lizards that involve epistatic interactions with self- and color-recognition loci [Sinervo et al. 2006b]).

Nevertheless, some genetic analyses are possible. The focus of these genetic analyses is on how the frequency of mating genotypes alters the dynamics. Laboratory crosses have been designed for all four mating systems presented in figure 2 (lizards: Sinervo et al. 2001, isopods: Shuster and Sassaman 1997, damselflies: Cordero 1990; tristylous: Fisher and Mather 1943). These crosses allow for construction of a genotype matrix of mating interactions, even if estimation of FD interactions among all genotypic combinations is impractical. Such genetic crosses can also reveal the existence of other loci that interact epistatically with morph determining loci (Shuster and Sassaman 1997, Sinervo et al. 2006b). Such epistatic loci are also predicted to evolve owing to mating system theory (Alonzo and Sinervo 2001, Shuster and Sassaman 1997).

In lizards, the factors governing self-recognition and settlement of genetically similar males reside at loci that segregate independently of the *OB* color locus. Moreover, the genetic factors governing settlement of *bb* males next to genetically similar *bb* males are shared between the sexes. These factors cause females to also mate with genetically similar males (Sinervo et al. 2006b). Assortative mating for self-recognition leads to a curious result. Given that self-recognition loci are shared between the sexes, females that carry alleles for self-recognition express a preference to mate with dyadic *bb* males involved in a cooperative relationship with a genetically similar *bb* male. However, females expressing self-preference need not possess a blue throat since the *OB* locus and self-recognition loci are on different linkage groups (Sinervo et al. 2006). Such assortative mating, driven by self-recognition loci, will have a powerful correlating effect on the mating system evolution of the RPS (see review). Description of these dynamics is beyond the scope of this user guide for FDS and payoffs. Interested readers should consult the model of socially mediated speciation presented by Hochberg et al. (2003).

In the case of tristylous, disassortative mating is so powerful that certain homozygous combinations are absent in the phenotypes (e.g. *SS--*, *--MM*). For example, flowers with *S* alleles do not cross-pollinate other flowers with *S* alleles (only *S*- \times *ss* matings are permissible; e.g. *S*-*M*- \times *ssM*- or *S*-*M*- \times *ssmm*). Thus, *SS* genotypes are largely absent from the multilocus genotype pool (see figure 2, bottom row, left panel). A selective consequence of such disassortative mating, which should affect all tristylous mating systems, is a buildup of deleterious or lethal recessives at modifier loci of dominant *S* allele expression. Alleles in the *SS* combination are rarely produced and any recessive modifiers of *S* alleles, which might be lethal, would rarely be exposed to purifying selection. Thus, disassortative mating has the curious effect of intensifying inbreeding depression from selfing or cross-pollination within floral types, resulting in a self-reinforcing process that further favors any other outcrossing mechanisms, which incrementally enhance self-incompatibility. Any genetic factors under correlational selection in tristylous

mating systems will also end up in linkage disequilibrium due to this disassortative mating.

In summary, even if genotype payoffs cannot be constructed, a first step is construction of the payoffs from mating interactions, which inform us about emergent properties that arise from correlational selection, non-random mating, and mating system evolution.

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