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Evolution is currently published by Society for the Study of Evolution.

FUNCTIONAL COMPLEXES AND ADDITIVITY IN PERFORMANCE: A TEST CASE WITH "FLYING" FROGS

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The origin and maintenance of morphological integration and of novel character complexes are common themes in recent literature (e.g., Cheverud, 1982; Wake and Larson, 1987; Zelditch and Carmichael, 1989; Wake and Roth, 1989), but the quantitative effects of such character complexes on performance have received much less attention (but see Shaffer and Lauder, 1985; Wainwright and Lauder, 1986; Andrews et al., 1987). The possible synergistic consequences for whole organism performance of coupling behavioral innovations with certain morphological features has been explored (e.g., Kingsolver, 1988; Emerson and Koehl, 1990), but the possibility that the individual elements of some morphological complexes may interact synergistically among themselves to affect performance has not. Nonadditive interactions among individual traits could contribute to the depth of the valleys between adaptive peaks of morphological variation and the steepness of the fitness surface that translates phenotypic variation into fitness variation (Lande, 1986 and references therein). Any such interactions would thus have important consequences for the rate of evolutionary origin and integration of complex design (e.g., Kingsolver and Koehl, 1985; Burger, 1986; Dawkins, 1986; Kingsolver, 1988). We present a quantitative study of the additivity of the performance capability in a suite of morphological characters associated with "flying" in frogs.

"Flying" frogs have evolved independently in both New and Old World families of treefrogs (Duellman, 1970; Liem, 1970). These frogs are not capable of powered flight but glide through the forest canopy. Old World "flying" species (family Rhacophoridae) are readily identified by a unique suite of derived morphological features: enlarged hands and feet, full webbing between all fingers and toes, lateral skin flaps on the arms and legs, and reduced weight per snout–vent length (Liem, 1970; Emerson, unpubl.).

These modifications of flying frogs reduce their "wing" loading (W, weight per projected area) relative to nonflyers and affect several aspects of their aerodynamic performance. Wind tunnel experiments (Emerson and Koehl, 1990) indicate that the flyer features listed above 1) increase the horizontal distance traveled (proportional to $[L/D]_{max}$, where L is lift and D is drag)

during a fall from a given height by a frog in the "flying posture" (described in Emerson and Koehl, 1990), 2) reduce the minimum speed (U_{min}) required for a falling frog to begin to glide, 3) increase the drag (D_{90}), and hence the time aloft of a parachuting frog, and 4) improve maneuverability by reducing the turning radius (inversely proportional to $[C_L/W]_{max}$ where C_L is the lift coefficient) achieved by a given banking angle. (The derivation of these performance variables, $[L/D]_{max}$, U_{min}, D_{90}, and $[C_L/W]_{max}$, is explained in Emerson and Koehl, 1990.) The present study examines whether individual elements of the flying character complex interact in an additive fashion in their effects on these four aspects of performance (e.g., if structure A increases performance by three units and if structure B increases it by five units, does the presence of A and B together increase performance by exactly eight units?).

METHODS

To evaluate the additivity or nonadditivity of the effects of a suite of morphological characters, we measured the aerodynamic performance of models of frogs in which we could manipulate individual elements of morphology independently. We used the body form of the nonflyer because other work had established there was no significant effect of body form on the performance variables (Emerson and Koehl, 1990). We assessed the effects of enlarged flyer hands alone by putting flyer hands on a model of a nonflying frog. Similarly, we examined the consequences of flyer feet alone and of flyer skin flaps alone. We also measured the effects of flyer hands, feet, and skin flaps when they occur together by simultaneously putting all these features on a model of a nonflyer. The components of our models were exact replicas of the special features of the flying frog *Rhacophorus nigropalmatus* and of the body of a closely related nonflying frog of the same weight, *Polypedates macrotis* (see Emerson and Koehl, 1990 for details of design). Models were made from a single individual of each species. The use of a single specimen is justified by noting that both species exhibit typically low coefficients of variation for morphological traits (<10%) while morphological differences between flying and nonflying forms are large (e.g., flyer surface area 1.5 times that of nonflyer of same body weight).

TABLE 1. Overall ANOVA results.

Performance variable	Source of variation	MS	F[†]	Coefficient of determination
$[L/D]_{max}$	Reynolds number	0.033	26.21	0.12
	Morphology	0.116	91.52	0.57
	Reynolds number * morphology	0.017	13.12	0.24
	Residual	0.001	—	—
U_{min}	Reynolds number	0.140	258.29	0.34
	Morphology	0.186	341.68	0.60
	Reynolds number * morphology	0.004	7.63	0.04
	Residual	0.001	—	—
D_{90}	Reynolds number	0.011	57,000	0.93
	Morphology	~0	2,051	0.05
	Reynolds number * morphology	~0	385	0.01
	Residual	~0	—	—
$[C_L/W]_{max}$	Reynolds number	170	220	0.31
	Morphology	239	309	0.58
	Reynolds number * morphology	11	14.6	0.08
	Residual	0.62	—	—

† All F-values significant at <0.0001.

We calculated the performance indices ($[L/D]_{max}$, U_{min}, D_{90}, and $[C_L/W]_{max}$) of models of defined morphologies from measurements of their lift and drag made in a wind tunnel (as described in Emerson and Koehl, 1990). The individuals used for models had flown in field performance tests, and we compared those results with values obtained from the models in the wind tunnel to verify the accuracy of our testing procedure (see Emerson and Koehl, 1990 for more details). Performance indices were transformed by taking the natural logarithms of ($[L/D]_{max}$ + 1), of U_{min}, of (D_{90} + 2), and of ($[C_L/W]_{max}$ + 10). Transformed variables were of comparable orders of magnitude. The analysis of data on the logarithmic scale technically examines nonmultiplicativity on the arithmetic scale of morphological variation. Hereafter we refer to nonadditivity on the logarithmic scale simply as nonadditivity; significant nonadditivity on the logarithmic scale of morphological variation also will exist on the arithmetic scale. The converse is not true: arithmetic nonadditivity is often eliminated by logarithmic transformation. Our tests on the logarithmic scale give us a conservative assay for nonadditivity on the arithmetic scale along which performance varies.

We examined the performance consequences of these manipulations on frogs of different sizes by varying the Reynolds number (Re). The Reynolds number, which represents the importance of inertia relative to viscosity for a particular fluid flow situation is given by

$$Re = UL/\nu$$

where U is velocity (e.g., the velocity of the air relative to a falling frog, hereafter called "airspeed"), L is a linear dimension (snout–vent length of a frog), and ν is the kinematic viscosity of the fluid (15.00×10^{-6} m² s⁻¹ for air at 20°C) (e.g., Vogel, 1981). Different flow situations for geometrically-similar bodies having the same Re are dynamically similar (i.e., the ratios of the velocities and of the forces at all corresponding points in the fluid are the same) (e.g., Daugherty and Franzini, 1977). An isometric doubling of the length (L) of a frog should have the same effect on coefficients of drag and

of lift as would doubling the airspeed (U), since either change doubles Re. Therefore we measured performance of each model at four realistic airspeeds (i.e., Re's) in the wind tunnel to explore the general consequences of body size. Two of the airspeeds we used were those recorded in live animal performance trials (4.5 and 6.1 m/s for the individuals we modeled). The lower airspeeds (1.1 m/s and 2.2 m/s) we used in the wind tunnel are analogous to body sizes of a smaller flying frog species *Rhacophorus pardalis* (SVL ≃ 45 mm) and of juvenile *Polypedates macrotis* and *Rhacophorus nigropalmatus* (SVL ≃ 25 mm). We will explore the details of size change to frog aerial performance elsewhere (Koehl and Emerson, in prep.).

We used a two-way analysis of variance to test for the presence of nonadditivity of the performance consequences of the morphological characters that typify 'flying' frogs and the possible dependence of any nonadditivity on the size of the frog. We did not add or remove each morphological feature in a complete $2 \times 2 \times 2$ design at each wind speed because it was impractical to perform three replicates at all 32 combinations of wind speed and morphology. We considered "morphological variation" as a single factor that was completely crossed with the four levels of wind speed and that had five levels within it: 1) control (nonflyer) morphology, 2) control with flyer hands, 3) control with flyer feet, 4) control bearing flyer skin flaps on arms and legs, and 5) control with flyer hands, feet, and skin flaps. We used three replications (assembling and testing a particular morphological configuration) at each of the 20 combinations of wind speed and morphology; equality of sample sizes allowed exact partitioning of the overall variance between the two factors and their interaction. The effect of each individual morphological feature and consequent additivity of features were tested by a priori contrasts among the levels of the overall main effect of "morphology" in the two-way analysis of variance (Rosenthal and Rosnow, 1985). The advantage of this approach is that it allows us to make the tests of direct interest to us (net nonadditivity of each wind speed) with a mini-

TABLE 2. Additivity at different Reynolds numbers.

Performance variable	Reynolds number (Re)	$F(1,10)$	P	Modified coefficient of determination*
$[L/D]_{max}$	5,668	9.99	<0.01	(0.27)
	11,337	0.60	NS	
	23,190	3.02	NS	
	33,680	3.24	NS	
U_{min}	5,668	5.03	~0.05	(0.03)
	11,337	51.24	<0.0001	(0.05)
	23,190	42.33	0.0001	(0.02)
	33,680	170.51	0.0001	(0.01)
D_{90}	5,668	0.78	NS	
	11,337	0.10	NS	
	23,190	77.41	0.0001	(<0.01)
	33,680	1,314.55	0.0001	(<0.01)
$[C_L/W]_{max}$	5,668	11.4	<0.007	(0.05)
	11,337	15.5	<0.003	(0.06)
	23,190	58.6	<0.0001	(0.04)
	33,680	377.0	<0.0001	(0.02)

* Proportion of the total effect of morphology that is attributable to nonadditivity.

mum amount of total replication. The disadvantage is that we cannot tell whether any nonadditive effects are due to strong pairwise interactions among any two features or full three-way interactions. We also cannot discover whether inability to reject the null hypothesis of additivity is due to a lack of any interactive effects or a directional interaction between two characters that is cancelled by the introduction of the third character.

There are two distinct sets of contrasts that can be used to make these tests. We can test the null hypothesis that flyer hands have no effect by comparing the model with flyer hands (level 2) to the control model (level 1). The advantage to this approach is that the statistical effect of each individual trait on performance variables has a clear, unambiguous interpretation. The problems with this approach are that the two-level contrasts for each trait are not mutually orthogonal and there is no contrast that will test additivity and simultaneously be orthogonal to all of the contrasts for every individual feature. An alternative procedure is to test the null hypothesis that flyer hands have no effect by comparing the average values of the two models that possess flyer hands (control + flyer hands, control + all flyer features) to the average values of the three models without flyer hands. Similar five-level contrasts can be synthesized for each of the other features. This approach has two disadvantages: interpretation of the statistically significant effect of any single feature is indirect and, like the previous approach, the contrasts for every trait are not mutually orthogonal (see Hays, 1973). However, this approach produces a five-level contrast for testing the null hypothesis of additivity that is orthogonal to every contrast for an individual trait and has a direct, unambiguous interpretation.

To illustrate interpretation of the five-level contrast for the test of additivity, let the average value of a performance variable for the control morphology be a. Now suppose the average value for control + flyer hands is b, that for control + feet = c, that for control + flaps = d, and that for control + all features = e. Under a null hypothesis of additivity, the effect of the

combination should equal the effect of hands plus the effect of feet plus the effect of flaps. The *effect* of a feature on performance is the increment or decrement in performance beyond the value of the control morphology, or the difference between the performance values with the feature and those without the feature (i.e., the control). Thus under additivity,

$$e - a = (b - a) + (c - a) + (d - a) \qquad (1)$$

which can be rearranged as a null hypothesis contrast

$$-2a + b + c + d - e = 0$$

or

$$2a - b - c - d + e = 0 \qquad (2)$$

"Reinforcing" nonadditivity is indicated if the left-hand side of (1) exceeds the right-hand side, which translates to a significant positive contrast in (2). Similarly, "weakening" nonadditivity is indicated by a significant negative contrast in equation (2).

RESULTS

Both morphology and Reynolds number (i.e., size) showed significant effects on all four performance variables (Table 1). Morphology accounted for most of the variance in the horizontal distance traveled ($[L/D]_{max}$), minimum glide speed (U_{min}), and maneuverability ($[C_L/W]_{max}$). However, Reynolds number (Re) was much more important in explaining the differences in drag while parachuting (D_{90}). Re showed a significant interaction with morphology for all four performance variables, indicating that the effects of different morphological configurations varied with the Re. We therefore analyzed the data for each Re separately to ascertain if nonadditive effects occurred only for some Re's and not others.

The importance of Re (i.e., body size) in producing nonadditivity varied among the different performance variables (Table 2). For horizontal traveling performance ($[L/D]_{max}$) there was nonadditivity only at the lowest Re's, with $[L/D]_{max}$ greater than that predicted from summing the effects of each of the morphological

variables. This meant that there was a significant, reinforcing nonadditivity for very small animals (SVL $\simeq 25$ mm) but not for large ones. Minimum glide speed (U_{min}) showed nonadditivity at all Re's. In this case, the U_{min} of the animal bearing flyer hands, feet, and flaps simultaneously was less than that expected on the basis of the effects of the individual components. Since the lower the minimum glide speed, the sooner a falling animal can begin to glide, the nonadditivity of U_{min} again resulted in improved performance over that calculated by summing the effects of each of the morphological characters. Parachuting performance (D_{90}), in contrast to $[L/D]_{max}$, showed nonadditivity only at the highest Re's (i.e., nonadditive effects on parachuting occurred only for very large animals, SVL $\simeq 80$ to 100 mm). The nonadditivity in this case produced frogs with poorer parachuting ability than expected from summing the effects of each of the individual components. Turning performance ($[C_L/W]_{max}$) showed significant nonadditivity at all Re's. The interaction effect was reinforcing in that maneuverability of animals bearing flyer hands, feet, and flaps simultaneously exceeded expectation based on the effects of the individual morphological features.

These results have interesting ramifications both for the specific case of the evolution of flying frogs as well as the more general issue of the evolution of morphological novelties. Both the New World flying frog, *Hyla miliaria,* and the Old World flyer, *Rhacophorus nigropalmatus,* exceed 100 mm in snout–vent length and are among the largest members of their respective families (Duellman, 1970; Inger, 1966). If time aloft while parachuting or horizontal gliding distance were the most important performance variables for flying frogs, the negative nonadditive interaction of D_{90} with large size and the positive nonadditive effect of small size on $[L/D]_{max}$ would argue against expecting efficient flying frogs to be as large as these two species. However, wind tunnel experiments (Emerson and Koehl, 1990) show that the morphological features and behavioral posture of flying frogs 1) exert their largest positive effect on maneuverability ($[C_L/W]_{max}$) and, 2) depending on the posture of the frog, can actually decrease the horizontal traveling performance ($[L/D]_{max}$) relative to that of a nonflyer. Maneuverability shows positive nonadditivity over all size ranges. Therefore, although the nonadditive effects play a significant role in enhancing certain performance values, the presence or absence of such nonadditive effects in frogs of different sizes do not offer any insights into why flying frogs are unusually large in size.

If a complex of characters occurring together improves performance either less or more than expected from the sum of their effects acting individually, such nonadditivity can play a major role in guiding the rate of evolution of the complex. The rate of evolutionary change is related to the steepness of the fitness surface (Fisher, 1930; Lande, 1979). Depending on the relationship between performance and fitness (Arnold, 1983, 1986), the nonadditive effects of a morphological complex on performance could alter the steepness of the fitness function. Positive nonadditive changes that improve performance could, presumably, yield a positive feedback that would not only result in rapid evolutionary change but also favor the developmental integration (Cheverud, 1982) of the features producing

the nonadditive effects, that is reinforce the integrity of the "character complex." In contrast, nonadditive effects of a group of characters that decrease performance when they occur together may serve to force evolutionary change away from that phenotype.

Unfortunately we have no information on the form of the relationship between performance and true absolute fitness in "flying" frogs. We can envision only one way in which the translations from performance to fitness might cancel the nonadditive translation from morphology to performance that we have found. If the performance–fitness translation was nonlinearly concave (convex) when the morphology–performance translation was convex (concave), then the two translations would produce an approximately linear relationship throughout the range of morphological variation we examined. Such an effect would have to include all the performance traits and exactly mirror each morphological trait's contribution to performance in order to cancel fully the nonlinear effects we suggest.

On the genotypic level the most pronounced nonadditive effects appear to occur under a regime of strong, directional phenotypic selection (Travis and Mueller, 1989). The data on flying frogs suggest a possible analogous situation for nonadditive phenotypic effects. It may be that the pattern of relatively rapid origin for character complexes that are considered to be evolutionary innovations is related in part to the degree of nonadditivity of the constituent traits in creating enhanced performance values for the whole organism. Our results on flying frogs provide clear empirical support for these ideas.

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Corresponding Editor: R. B. Huey