Morphogenetic basis for phenotypic differences in hydroid competitive behaviour

Leo W. Buss* and Richard K. Grosberg†

* Departments of Biology and Geology and Geophysics, Yale University, New Haven, Connecticut 06511, USA
† Department of Zoology, University of California, Davis, California 95616, USA

FOR many plants, fungi and sessile invertebrates, the availability of habitable space often limits their size, reproductive output and survival. Intraspecific and interspecific variation in the ability to compete for space is common[1–3], but the way in which competitive ability is regulated remains largely unknown. We describe here a system of intraspecific competition in the hydroid *Hydractinia symbiolongicarpus* in which the principal mechanisms underlying variation in competitive ability have proven amenable to experimental analysis. Competitive interactions can be aggressive, involving the induction of a specialized fighting organ called a hyperplastic stolon, or non-aggressive, in which no hyperplastic stolons are produced. Colonies display continuous variation in the ontogenetic appearance of tissues that differ in their competence to be induced to produce hyperplastic stolons. We find that the outcome and kind of competitive interaction between strains are predictable given knowledge of their morphologies. In this hydroid, complex competitive behaviour arises from a coupling of discrete morphogenetic potential of differing tissue types with continuous variation in the ontogenetic and astogenetic appearances of these tissues.

Colonies of *H. symbiolongicarpus* are commonly found encrusting gastropod shells inhabited by pagurid hermit crabs[4]. Intraspecific competition occurs at high frequency in natural populations[5–7]. Encounters between allogeneic colonies (that is, interactions between unrelated genotypes) result in one of two different rejection responses[8–11]. The first response—passive rejection—is characterized by the secretion of a fibrous matrix by both colonies[10], and is accompanied by a virtual cessation of growth along the border between the two colonies (Fig. 1*a*). Aggressive rejection (Fig. 1*b*), by contrast, is mediated by the induction of a specialized organ of aggression, the hyperplastic stolon[10–14]. On encountering foreign tissues, stolons hypertrophy as a result of widespread movement of nematocytes into the stolon tip and subsequent nematocyst discharge[10,14] (Fig. 1*d*). Nematocyte recruitment and discharge continues until either the foreign tissue is destroyed or until the hyperplastic stolon is destroyed by a similar hyperplastic response from its neighbour.

Colonies of *H. symbiolongicarpus* display considerable variation in gross colony morphology during early ontogeny[15,16]. Colonies grow by the asexual iteration of feeding polyps, by the radial expansion of a basal ectodermal mat, and by the peripheral extension of tubular stolons that ramify and anastomose over the substratum. Colonies range from completely stolonless (Fig. 2*a*) to highly stoloniferous (Fig. 2*b*). Experiments in which clonal replicates of each of 14 different *H. symbiolongicarpus* strains were reared under constant conditions in the laboratory (that is, 'common garden experiments'), revealed significant variation among the strains in the relative rates of mat, polyp, and stolon production (Table 1).

In addition to ontogenetic variation in relative rates of mat, polyp, and stolon production, there is considerable variation in the form of mat tissue at the colony periphery. The ectoderm of the mat tissues can consist of unfused tubes (Fig. 2*c*) similar to that found in stolons; alternatively, the ectoderm of adjacent gastrovascular canals can be laterally fused into a continuous sheet. In some colonies, all mat tissue consists of fused ectoderm, whereas in other colonies, there is a central region of fused mat surrounded by a peripheral region of unfused mat. This region of unfused mat is called the astogenetic zone[17]. Thus, a zone of astogeny is entirely absent in some colonies, whereas an astogenetic zone of varying width occurs in other colonies. As for ontogenetic variation, common garden experiments suggest that there is a substantial genetic component to variation for both the presence or absence of an astogenetic zone, as well as for the width of the astogenetic zone (Table 1).

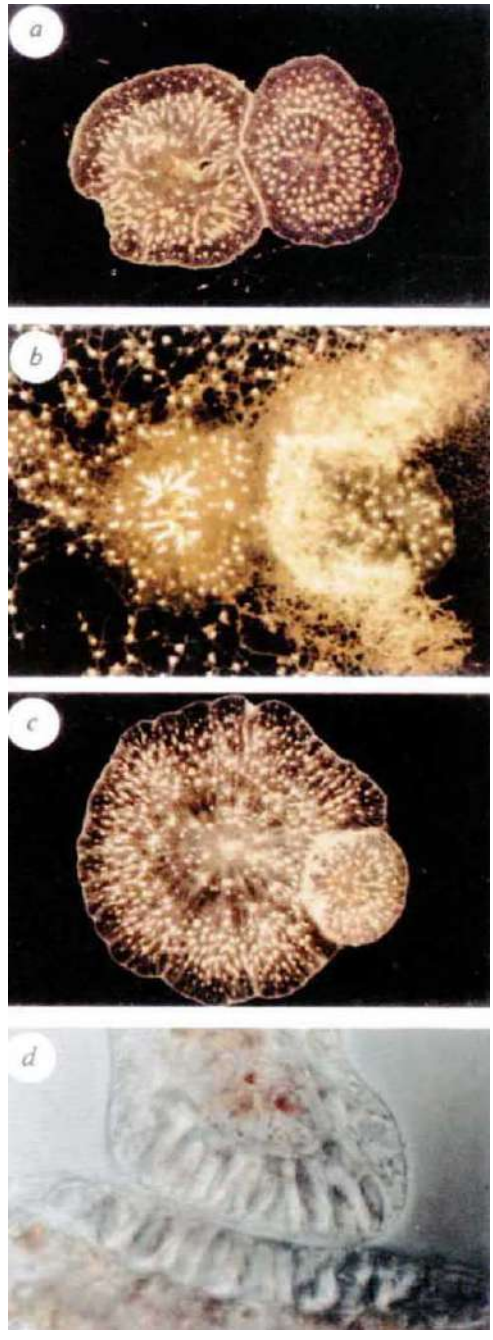

FIG. 1 *a*, A passive rejection between two stolonless *Hydractinia* colonies. *b*, An aggressive rejection between two stoloniferous *Hydractinia* colonies. Note the extensive development of hyperplastic stolons separating the two colonies. *c*, An interaction between two stolonless colonies escalating from an initially passive response to an aggressive response. Note the development of hyperplastic stolons along the margin of the larger colony in contact with the smaller colony. *d*, A Nomarski interference micrograph of contact between allogeneic stolons showing the array of nematocysts at the point of contact (photograph by R. Lange and G. Plickert).

As a consequence of ontogenetic and astogenetic variation in colony morphology, competitive encounters between colonies of *H. symbiolongicarpus* can involve any combination of three different tissues: stolons, unfused ectodermal mat (termed UM), or fused ectodermal mat (termed FM). Allogeneic encounters between stolons and any other tissue type result in the induction of a hyperplastic response[8]. A series of grafting experiments clarifies the morphogenetic potential of the two types of mat tissues. Reciprocal transplants of central FM tissues from one colony into contact with either the central FM ($n = 15$) and peripheral UM ($n = 15$) of an allogeneic colony yielded a passive response in all cases. By contrast, grafts from the peripheral UM of the same colonies in contact with either central FM ($n = 15$) or peripheral UM ($n = 15$) initially showed a passive response, followed within 1–3 weeks by the induction of an aggressive response. This aggressive response began as stolons emerged from the acellular matrix at the periphery of the mat; these subsequently differentiated into hyperplastic stolons. Thus, the two forms of rejection—passive and aggressive—are determined by differing morphogenetic potentials of the tissue types coming into contact with one another.

The discrete developmental potentials of stolon and mat tissues, when coupled with strain-specific variation in the pattern and rate of appearance of these tissues, potentially leads to a complex array of competitive behaviours for this simple diploblastic organism. Stoloniferous colonies should behave aggressively (Fig. 1*b*) and stolonless colonies with fused mat should not (Fig. 1*a*). But a range of more complex behaviours are also possible. For example, stolonless colonies with unfused mat should initially behave non-aggressively, only to escalate interactions to involve aggression (Fig. 1*c*). Conversely, an aggressive response can be de-escalated if, during the course of a competitive encounter, the central fused mats of one, or both, colonies come into contact.

Competitive ability and outcome should thus be determined by the ontogenetic and astogenetic state of the competing colonies. We examined the outcome of pairwise size-symmetric competitive interactions between colonies in laboratory culture. We established these interactions by explanting equally sized explants of two colonies, separated by ~0.5 cm, onto glass microscope slides. These interactions were monitored roughly fortnightly until one colony had eliminated the other, or in cases of prolonged interactions, for nearly six months.

Stoloniferous versus stoloniferous phenotypes: We established replicated competitive interactions between seven stoloniferous

TABLE 1 Common garden experiments

Series	Tissue type	Degrees of freedom	*F*	Significance level (*P*)
Stoloniferous	Polyps	18, 78	23.43	0.001
	Mat	18, 81	17.59	0.001
	Stolon	18, 81	21.93	0.001
Stolonless	Polyps	24, 136	8.15	0.001
	Mat	24, 136	14.30	0.001
	Astogeny	12, 66	12.27	0.001

Analysis of variance for the effects of colony genotype on growth trajectories of polyp, mat, stolon and width of the astogenetic zone in two common garden experiments. These experiments were established by clonally propagating field collected colonies. A portion of ectodermal mat containing 3–5 feeding polyps (that is, an explant) was surgically removed, placed on a glass microscope slide, and held in place with a loop of thread. Explants attached to slides within 1–7 days and threads were removed. Stock colonies established in this manner were maintained in recirculating aquaria and fed to repletion daily with brine shrimp nauplii. Common garden experiments were established from stock colonies in two series. In the first series, five replicates of each of seven stoloniferous strains were explanted onto glass slides, maintained as were stock colonies, and their growth monitored at roughly 2-week intervals by macrophotography. In the second series, the growth of 4–6 replicates of each of seven stolonless strains was monitored. For each sampling interval, the number of polyps and the area of mat was calculated from photographs. In addition, stolon area (that is, the area prescribed by connecting the endpoints of adjacent stolon tips) was calculated for all stoloniferous strains and the width of the astogenetic zone measured for all stolonless strains. Mat and stolon area were calculated by tracing the outline onto a digitizer interfaced to a microcomputer. Log-transformed data were analysed as a two-factor design with repeated measures on one factor[19]. The main effects are strain and day, where day is treated as a categorical variable and each replicate was observed under all levels of day. When testing between-strain differences in growth rate, the relevant null hypothesis is that there is no strain-by-day interaction. This interaction mean square is tested against the day-by-replicate within-strains error mean square. Common garden experiments of this sort provide an estimate of broad-sense heritability[20]. Quantitative genetic analysis of variation in colony morphology reveals low additive to non-additive genetic variation for shape characters[21].

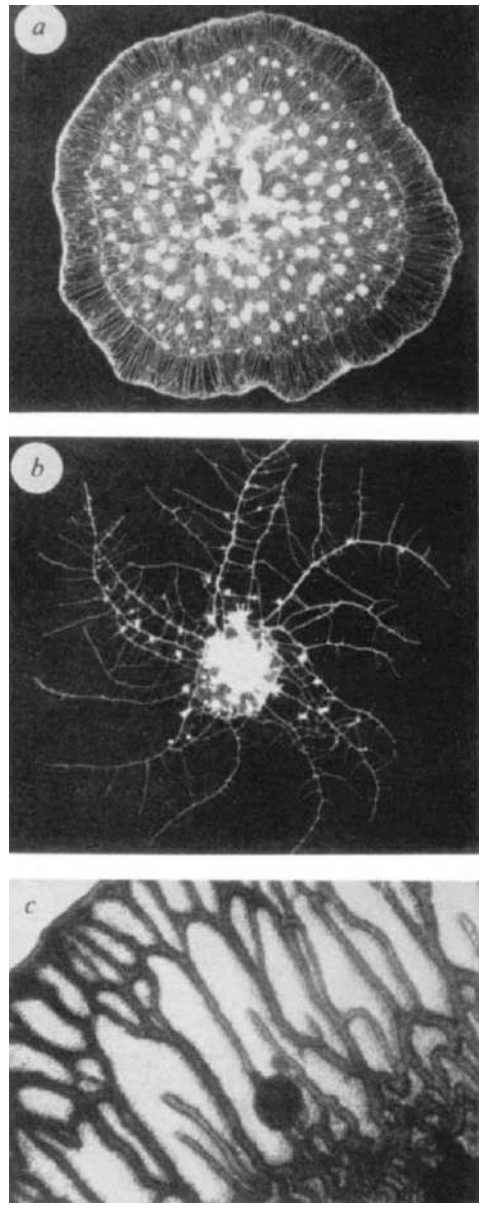

FIG. 2 *a*, A stolonless colony. *b*, A stoloniferous colony. *c*, The astogenetic zone of a UM colony, showing peripheral gastrovascular canals embedded within a transparent matrix. Note that beyond this marginal zone, gastrovascular canals become more dense. In central regions of the colony, the ectoderm of adjacent canals fuse to form a continuous sheet (not shown).

strains in 20 of the 21 possible pairwise combinations and observed these interactions for up to 173 days. All pairwise interactions ($n = 86$) were characterized by the reciprocal development of hyperplastic stolons. In 95% of all interactions one colony killed the other within 37–150 days. Interactions between stoloniferous colonies were transitive, with rapidly growing strains overcoming the slowly growing strains in all pairwise combinations (Fig. 3*a*).

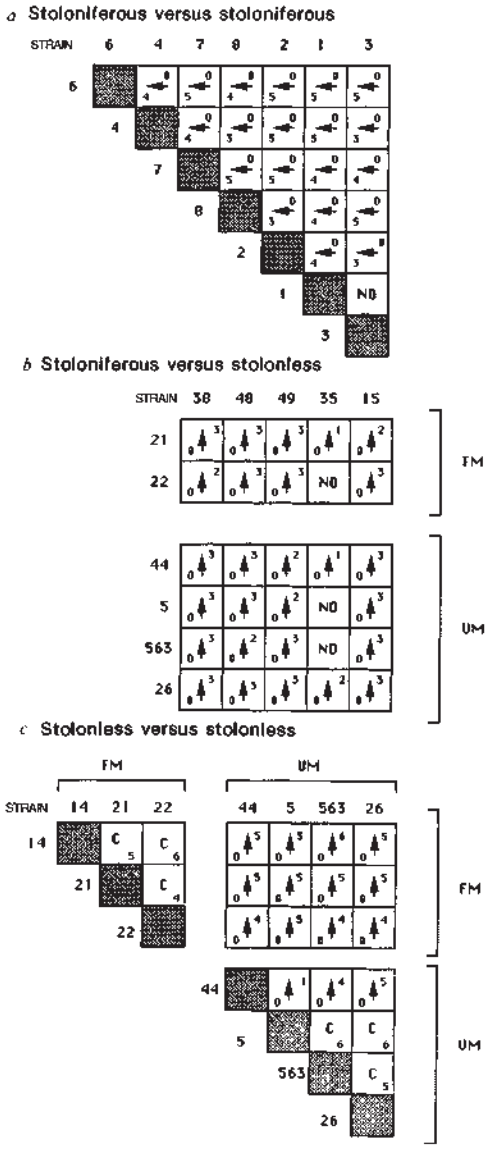

FIG. 3 Results of pairwise competitive interactions between hydroid colonies differing in ontogeny and astogeny. *a*, Interactions between seven stoloniferous strains. Each cell in this matrix represents interactions between the strain on the left and the strain above. The arrow points toward the strain which eliminated or was judged to be in the process of eliminating the other at the termination of the experiment. The number in the bottom left-hand corner of each cell indicates the number of replicates won by the colony on the left, and the number in the upper right-hand corner indicates the number of replicates won by the colony above. Strains are ordered left to right by their relative growth rates as measured in common garden experiments (that is, strains were ranked for log-transformed growth rate for each tissue type and the sum of the ranks for all three tissue types used to order colonies). ND, no data. *b*, Interactions between six stolonless strains (rows) and five stoloniferous strains (columns). C, coexistence. Strains are categorized by their astogenetic state. Number of replicates indicated in bottom left-hand corner of each cell. All other notations as in *a*. *c*, Interactions between seven stolonless strains. All other notations as in *b*.

Stolonless versus stolonless phenotypes: Replicated interactions between seven stolonless strains were established for three UM and four FM strains in all pairwise combinations and observed for up to 113 days. No evidence of aggression was observed in any of the three pairwise combinations involving FM strains ($n = 15$). All FM colonies in competition with other FM colonies coexisted for the entire experimental period (Fig. 3*b*). In contrast to the results of FM versus FM interactions, all pairwise combinations between FM and UM strains ($n = 48$) resulted in either the elimination of the FM colony (44%, $n = 21$) or in the loss of considerable tissue mass by the FM colony at the termination of the experiment (Fig. 3*b*). In each case, the UM colony began the interaction with a passive response, followed after 1–3 weeks by an aggressive response as stolons emerged from the acellular matrix at the edge of the mat. These emergent stolons soon became hyperplastic. By comparison, no FM colonies behaved aggressively.

Interactions between UM strains and other UM strains resulted in coexistence in three combinations ($n = 17$) and prolonged aggression in three other combinations ($n = 10$) (Fig. 3*b*). In all cases of coexistence, an initially passive response was followed in 1–3 weeks by aggression. After an aggressive interval of varying duration, a passive response spread along the interactive margin as the central FM of both colonies came into tissue contact. All cases of prolonged aggression occurred in interactions involving a single slowly growing strain (Fig. 3*b*). This strain was either eliminated, or in the process of being eliminated, before the central FM regions came into contact.

Stolonless versus stolonliferous phenotypes: Interactions between five stoloniferous strains and six stolonless strains (2 FM, 4 UM) were established in 27 of the 30 possible pairwise combinations and observed for 45 days. In all interactions involving a stolonless FM colony ($n = 23$), the stoloniferous colony was eliminating the FM colony in all replicates at the termination of the experiment. All stoloniferous colonies produced hyperplastic stolons and all FM colonies did not. In all interactions involving a UM colony ($n = 48$), the stoloniferous colony produced hyperplastic stolons on contact with the UM colony, whereas the UM colony initially displayed a passive response. Eventually UM colonies displayed a hyperplastic response; nevertheless, the stoloniferous colony was overgrowing the UM colony in all interactions at the termination of the experiment.

These experiments demonstrate that competitive ability is a simple deterministic function of growth morphology in this hydroid. Despite their simplicity, hydractiniid hydroids display a repertoire of aggressive behaviours like those of behaviourally sophisticated organisms[18]. Some colonies always behave aggressively, others never behave aggressively, and yet others can switch their behaviour from aggression to non-aggression (or vice versa). The capacity to display a diverse array of competitive behaviours is attributable to the central part played by the developmental processes of induction and competence in this system. Here competitive ability is not coded as a series of discrete alternatives; indeed morphological variation seems to be continuous. This variation, nevertheless, results in discrete competitive phenotypes because tissue types differ in their competence to be induced to form hyperplastic stolons. Differing competence defines a series of discrete behaviours for differing tissues. As such, an enormously complex range of competitive phenomena can occur as a consequence of discrete morphogenetic potential of differing tissue types whose ontogenetic appearance displays continuous variation. □

Received 17 May; accepted 24 October 1989.

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ACKNOWLEDGEMENTS. We thank M. Bertness, N. Blackstone, J. Bonner, J. Buss, D. Carlson, R. Crozier, C. Cunningham, A. Ellison, D. Green, G. Hartnett, N. Knowlton, C. McFadden, A. Shenk, H. Waldeman, J. R. Vaisnys and P. Yund for discussions of this work, and T. Jenkins, R. Lerner, M. Marthas, J. Taschner and H. Waldman for technical assistance. This work was supported by the NSF

choice[3,10,12]. In all studies to date in which female mate preferences based on call frequency are expressed, tuning of the peripheral auditory system predicts the preference by indicating frequencies that would most strongly stimulate the auditory system. There are no reported examples of any mechanisms overriding this peripheral bias. We tested whether differences in auditory stimulation could account for interspecific mate choice by obtaining acoustically-evoked multiunit activity from the torus semicircularis (inferior colliculus) to estimate the frequency sensitivities of the amphibian and basilar papillae[3,13]. Resulting audiograms were averaged and truncated below 1.5 kHz to yield a mean basilar papilla audiogram (Fig. 1). We then determined the Fourier spectrum (energy versus frequency)[3] of chucks from 54 males randomly sampled from a population on Barro Colorado Island, Panama[4–8] (Fig. 1). The mean dominant (peak energy) frequency of the chucks (2.55 kHz) was higher than the mean best frequency of the basilar papilla (2.13 kHz).

Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*

Michael J. Ryan*, James H. Fox†, Walter Wilczynski† & A. Stanley Rand‡

* Department of Zoology and † Department of Psychology, University of Texas, Austin, Texas 78712, USA
‡ Smithsonian Tropical Research Institute, Balboa, Panama

THE sensory bases of species and population mate preferences are well known[1–3]; in frogs properties of the female auditory system influence such preferences[2,3]. By contrast, there is little understanding of how sensory characteristics could result in sexual selection within a population. One possible mechanism is that females are more sensitive to male courtship signals that deviate from the population mean. We document this mechanism in the frog *Physalaemus pustulosus*. Female basilar papilla tuning is biased toward lower-than-average frequencies in the 'chuck' portion of the male's call, explaining female preference for the lower-frequency chucks produced by larger males. The tuning does not differ between *P. pustulosus* and its close relative *P. coloradorum*, a species in which males never evolved the ability to produce chucks; thus the female tuning evolved before the chuck and therefore the chuck played no role in the evolution of the preference. This allows us to reject two popular hypotheses for the evolution of this female preference (runaway sexual selection and natural selection) in favour of a third: sexual selection for sensory exploitation.

Male *P. pustulosus* produce an advertisement call consisting of a 'whine', which is necessary and sufficient for species recognition, followed by 0–6 'chucks', which increase the attractiveness of the call to females[4–8]. Chucks have a fundamental frequency of ~220 Hz with 15 harmonics. Most of the energy ($\bar{X} = 90\%$, s.e. = 0.02, $N = 110$) is above 1.5 kHz, and thus mainly stimulates basilar papilla receptors rather than the lower-frequency amphibian papilla receptors[9,10]. In phonotaxis experiments, females prefer synthetic calls having chucks with lower fundamental frequencies. Larger males produce lower-frequency chucks and have greater mating success in nature. Thus preferential female phonotaxis generates sexual selection[4–8].

This preference could result from the upper harmonics of lower-frequency calls eliciting greater neural stimulation of the high-frequency basilar papilla receptors due to differences in peak frequency or harmonic structure[11]. Similar mechanisms appear to guide interspecific and interpopulational mate

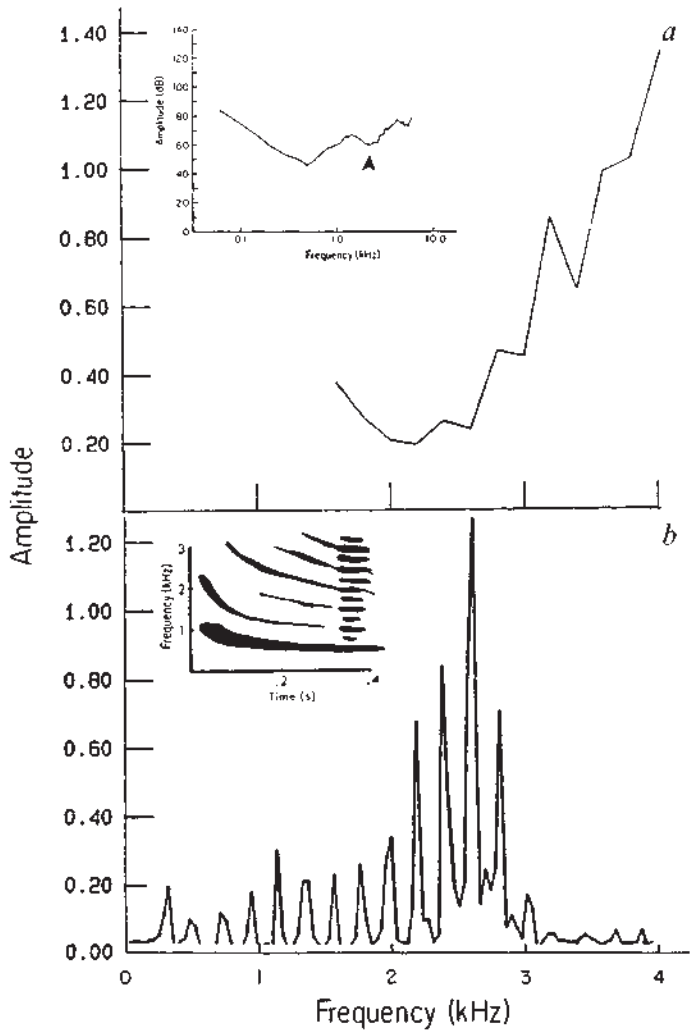

FIG. 1 *a*, The mean audiogram of the basilar papilla of *P. pustulosus* derived from five individuals. Audiograms represent thresholds as a function of frequency, determined for sinusoidal, closed-field stimuli using 1–2 MΩ glass electrodes. The truncation of the audiogram below 1.5 kHz to eliminate influences of amphibian papilla neurons and the slight broadening of the tuning curve resulting from averaging biases the results toward the null hypothesis. Insert, audiogram from a single frog; basilar papilla best frequency is marked by arrow. Male and female audiograms did not differ. *b*, Representative Fourier spectrum of a chuck. Insert, sonogram of a whine plus a chuck.