Cross-fostering alters advertisement vocalizations of grasshopper mice (*Onychomys*): Evidence for the developmental stress hypothesis

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HIGHLIGHTS

- Cross-fostering experiments to examine vocal learning may cause unintentional stress.
- Cross-fostered mice suffered developmental delays and emitted higher frequency calls.
- Call frequency did not match tutors, refuting vocal learning and supporting stress.

ARTICLE INFO

ABSTRACT

Nutritional stress can have lasting impacts on the development of traits involved in vocal production. Cross-fostering experiments are often used to examine the propensity for vocal learning in a variety of taxa, but few studies assess the influence of malnourishment that can occur as a byproduct of this technique. In this study, we reciprocally cross-fostered sister taxa of voluble grasshopper mice (genus *Onychomys*) to explore their propensity for vocal learning. Vocalizations of *Onychomys leucogaster* did not differ between control and cross-fostered animals, but cross-fostered *Onychomys arenicola* produced vocalizations that were higher in frequency in a direction away from tutors. These same animals exhibited a transient reduction in body mass early in development, indicative of malnutrition. Our findings simultaneously refute vocal learning and support the developmental stress hypothesis to highlight the importance of early ontogeny on the production of vocalizations later in life.

1. Introduction

Vocal learning is among the most remarkable adaptations in the animal kingdom. The ability to modify spectral content of acoustic signals in response to auditory experience has evolved independently in multiple lineages of birds (songbirds, parrots, hummingbirds) and mammals (humans, cetaceans, bats, elephants, and pinnipeds; [24,25]). Although the developmental and neural bases of vocal learning are best studied in oscine songbirds [5,25,35], recent evidence indicates that mice may possess some capacity to modulate spectral output of ultrasonic vocalizations (USVs; [21]) depending on auditory feedback [2]. As representatives of nearly 40% of mammalian diversity [50], the potential emergence of vocal learning in rodents provides important opportunities to improve our understanding of the mechanisms underlying this unique trait [51].

Cross-fostering experiments are a classic paradigm used to assess the learned and genetic bases of vocal behavior in a variety of taxa [6,8,38]. In laboratory mice, strain-specific vocal signatures persist in cross-fostered individuals [26]. Similarly, spectral features of isolation vocalizations are dependent on pup genotype following embryo transfer [1,52]. Such findings corroborate experiments supporting strong innate control of USV production in mice that are genetically deafened [19,29] or lacking a cerebral cortex [20]. However, Arriaga et al. [2] reported spectral convergence of USVs between different mouse strains that were cross-housed, indicating a degree of vocal plasticity previously unappreciated. Further studies on additional mouse strains and species are thus needed to determine the factors influencing the propensity for vocal learning.

One potential pitfall of cross-fostering experiments may be that offspring development is perturbed in fostered animals due to

malnutrition [22,31,41]. In birds, stress imposed by malnutrition experienced early in life impairs development of neural song control nuclei, resulting in reduced song complexity via decreased song learning ([36]; [28] and references therein). Developmental stress could also modify structures involved in sound production at the source (larynx or syrinx) or filter (vocal tract; [10,48]) independent of vocal-learning capacity [7]. Reductions in the length of vocal folds or vocal tracts are predicted to increase fundamental and formant frequencies, respectively, based on principles of acoustic allometry [15,16]. Yet, few studies have explored this hypothesis.

In this study, we simultaneously explore the role of vocal learning and developmental stress on vocal production in grasshopper mice (*Onychomys*). Grasshopper mice are carnivorous, territorial rodents that inhabit semi-arid desert, prairie, and scrub vegetation throughout the central and southwestern United States and northern Mexico [3,14,30]. Both sexes emit audible (<16 kHz), long-distance advertisement vocalizations used in mate attraction and territorial advertisement ([18, 33]; Fig. 1.). Northern grasshopper mice (*Onychomys leucogaster*) are larger (35 g; below) and produce consistently lower frequency vocalizations (~12 kHz) than Chihuahuan grasshopper mice (*Onychomys arenicola*; 22 g, ~15 kHz). We conducted a cross fostering experiment to examine if early auditory experience and/or malnutrition influences species-specific vocalizations. If the genus is capable of vocal learning, we predicted that vocalizations of cross-fostered animals would be more similar to their foster parents than their genetic parents. If vocalizations are innate, we predicted that vocalizations of cross-fostered animals would be more similar to their genetic parents than their foster parents. Nutritional stress—as indicated by a reduction in pup growth during early development— is expected to increase frequencies independent of vocal learning.

2. Materials and methods

2.1. Animals

Individuals of both species were wild-captured near Tank Mountain (31°48′46.90″N 108°48′49.90″W) in the Animas Valley, New Mexico, USA and transferred to animal facilities at Cornell University, Ithaca, NY. Mice were caged as same-species pairs for breeding, maintained on a 14:10 light/dark cycle (21 ± 2 °C), and provided rodent chow and water ad libitum. We visually inspected females for pregnancy daily, and thereafter monitored for parturition every 8 h. Litter sizes used in this study were consistent in each species (*O. arenicola* = 4/litter, *O. leucogaster* = 5/litter). We reciprocally cross-fostered two pups from each litter born within 8 h of one another. Control mice were handled in the same manner as fostered pups but returned to their biological parents. Thus, all litters had a combination of control and fostered animals together with both parents. However, only one control and one cross-fostered animal were recorded per litter. Focal cages were transferred to semi-anechoic cubicles in a separate room from the animal colony so that they were exposed only to calls of their parents or foster parents. Under laboratory conditions, adult males in reproductive condition typically call nightly. While we did not monitor the number of vocalizations emitted by parents, a researcher confirmed call production each night with visual observations. Pups were weaned at 30 days, housed with animals of the non-cross fostered controls of the different species until the onset of aggression and sexual maturity (emergence of *swollen testes* and vaginal perforation) around six weeks [39,46,47], and then separated until the initiation of call production around 10–12 weeks. Animals were exposed to tutors for the entirety of the experiment. Although we designed a paired experiment, unequal sample sizes reflect occasional rejection of pups from both control and foster groups. Thus, only a subset (*n* = 4/treatment) of animals were weighed at birth, 15 days, 30 days, and 80 days. Design and sample sizes are in Fig. 2.

2.2. Acoustic recording

Individual animals in their home cage were placed within a semi-anechoic sound cubicle for overnight (10 h) recording. Tutor males were also recorded overnight following weaning of pups. We used 1/4″ microphones (Type 40BE, G.R.A.S.) connected to preamplifiers (Type 26 CB, G.R.A.S.) to obtain acoustic pressure recordings. Playback of a 13 kHz pure tone from different areas of the cage within the cubicle
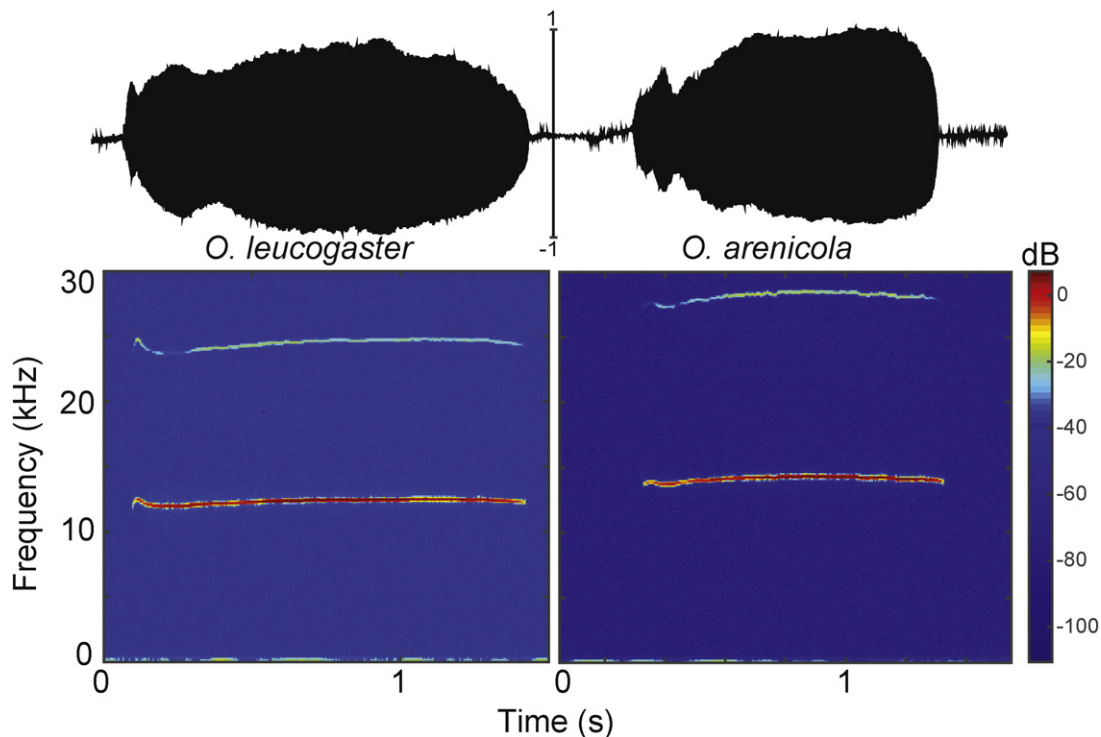

Fig. 1. Representative waveforms and spectrograms (FFT = 1024, Hanning window, 50% overlap) of *O. arenicola* and *O. leucogaster*.