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Infant boys are more vocal than infant girls

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Female humans appear to have an advantage in language, from early childhood through late adulthood, reported to include a larger vocabulary, more complex utterances, greater expressive language, and better verbal and pragmatic language comprehension [1]. Wakeful infants produce 'protophones' — precursors to speech that include vowel-like sounds, squeals, and growls — at a rate of four or five utterances per minute, more than five times the rate of crying, throughout the first year [2]. The massive number of protophones is in itself surprising, but equally surprising, given the presumed female language advantage, we found that, in the first year, boys produced 24% more protophones than girls. This sex bias was true of infants either at high risk (HR) or low risk (LR) for autism. Both genetic and cultural factors may be involved in this bias, and additional research is clearly called for to investigate the origins of the strong tendency of infants to produce protophones and the unexpected tendency for boys to do so to a greater extent.

Figure 1A shows the highly significant result favoring boys (*t*-test, $p < 0.0001$) with an effect size (Cohen's $d = 0.89$) more than four times larger than that typically reported for female language advantage [3]. Both HR and LR boys' protophone rates were significantly higher than girls' (HR, $p < 0.005$, boys 27% higher, $d = 1.02$; LR, $p = 0.01$, boys 21% higher rate, $d = 0.78$). Figure 1B displays rates for infants grouped by age, boys showing higher rates at all ages. Figure 1C,D shows results for HR and LR infants, with higher rates in boys at all ages. Generalized Estimating Equations (GEE) tested the Age, Sex, and Risk factors, revealing a Sex effect ($p < 0.0001$) and an Age by Sex interaction ($p < 0.05$), corresponding to a decreasing difference between boys and girls across

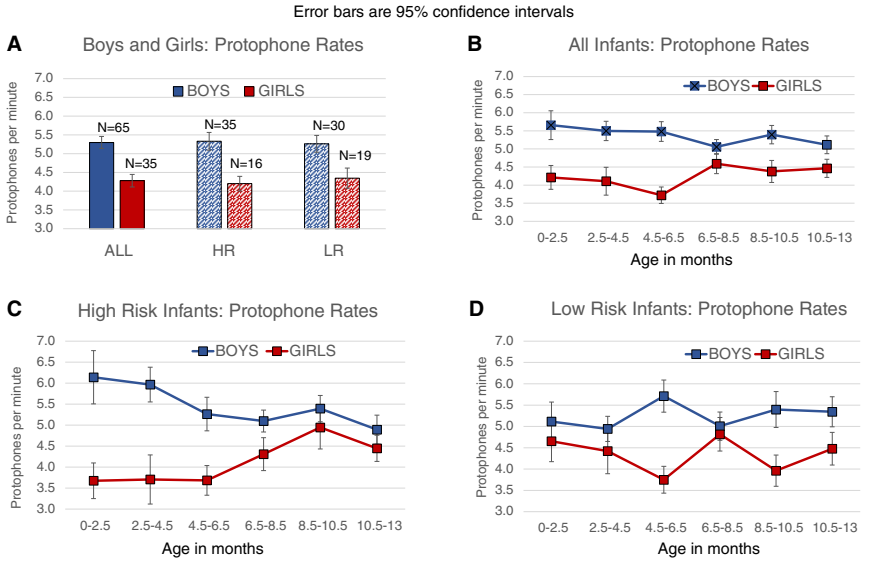

Figure 1. Protophone rates in boys and girls.
(A) 65 boys produced about one protophone per minute more (approximately a thousand more protophones per day) than 35 girls ($p < 0.0001$). The difference favoring boys applied significantly to both infants at high risk (HR) for autism and infants at low risk (LR). Error bars are 95% confidence intervals. Data pertain only to infants who were awake. (B,C,D) Age analysis revealed that both HR and LR boys produced more protophones at all ages across the first year.

ages (Figure 1B), a pattern driven mostly by the diminishing difference across Age in the HR infants (Figure 1C). Thus, contrary to expectations, protophone rate was higher in boys than girls across the first year, with greatest difference at the earliest ages.

We wondered if the higher protophone rate of the boys would correspond to more rapid development of advanced protophones, namely canonical babbling — baba, mama, and so on — which begins at approximately seven months and involves well-formed syllables that can be used in words [4]. The canonical babbling ratio (CBR) is the number of canonical syllables, such as [ba], divided by the total number of syllables an infant produces, including non-canonical syllables, usually vowel-like sounds. Notably, whereas deaf infants show no reduction in protophone rate, they are sharply delayed in onset and rate of canonical babbling [5]. So protophone rate and canonical babbling may be somewhat independent.

Indeed, boys had no advantage over girls in CBR (Figure S1 in the Supplemental Information), which increased as expected significantly for both sexes across Age ($p < 0.005$) and Risk (LR higher, $p < 0.05$). Thus canonical babbling, a scaffold for first word acquisition, showed no sex bias, but did show the expected increase with age as well as a higher CBR in LR infants, a finding consistent with prior reports of disruption in canonical babbling of infants with or at risk for autism [6].

We did not set out to study sex effects in speech-precursors, but the longitudinal research reported here afforded us the opportunity to reliably evaluate sex effects through extensive human coding at considerable sample size both of intensive longitudinal home-recordings and of infants. The infants were recorded all day and approximately monthly across the first year (65 boys, $M = 8.55$ all-day recordings; 35 girls, $M = 8.60$) using a miniature audio recorder, yielding ~6800 hours of recording. Twenty-one randomly-sampled five-minute segments from each recording were coded in real-time by a trained team, yielding >330,000 protophones and >50,000 cries. Coders were blind regarding infant age, sex, and risk status. Coding reliability was high, and discrepancies among coders were small with regard to the effect, indicating boys produced more protophones than girls (see Supplemental Experimental Procedures for methods details and demographics, and Table S1).

Cultural factors could contribute to sex differences in protophone rates. But we

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know of no comparative cross-cultural research on vocal rates of infant boys and girls nor on possible differences in caregiver speech to boys and girls across cultures. A non-significant tendency for caregivers to speak more to boys was seen in our data (see Supplemental Results), and other possible cultural factors could also influence sex differences in infant vocal rates (see Supplemental Discussion).

It is possible that the sex difference is not closely related to language *capability* — the CBR did not show a sex difference — but rather to a difference in *the tendency to vocalize*, perhaps owing to sex differences in motoric activity level in infancy [7]. Boys might be said to show higher *quantity* but not *quality* in protophone production. Another hypothesis can be formulated in conjunction with a proposed explanation for the high rate (thousands per day throughout the first year) of human protophone production in both sexes (see Supplemental Discussion). The protophones appear to be produced largely endogenously — they are most commonly *not* directed toward other speakers, occurring at a rate of approximately four per minute even when infants are alone [8]. Even infants born more than two months prematurely and still in neonatal intensive care produce prodigious numbers of protophones [2]. Furthermore, as noted above, there is no sign that deafness reduces protophone rates [5].

This audible endogenous motoric activity, usually produced by infants in comfort, might be motivated by its value as a fitness signal for the altricial human infant, competing for parental investment [9]. One might then suggest that evolution has led to boys signaling their fitness more frequently than girls because they are more vulnerable to death in the first year [10]. This fitness signaling hypothesis could be explored, for example, by correlating parental investment with infant protophone rates. We are, however, seeking other possible explanations for this unexpected sex difference in infant vocal rates (see Supplemental Discussion).

SUPPLEMENTAL INFORMATION

Supplemental Information includes one supplemental figure, one supplemental table, Supplemental Experimental Procedures, Supplemental Results, Supplemental Discussion, and Author Contributions and can be found with this article online at https://doi.org/10.1016/j.cub.2020.03.049.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Establishment of homozygous knock-out sea urchins

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Sea urchins have played important roles in cell and developmental biology research for more than a century [1,2]. However, due to their long breeding cycle, it has been recognized that it is not realistic to introduce genetic methods into sea urchin research. Here, we introduce a new sea urchin model species, *Temnopleurus reevesii*, and demonstrate the successful production of not only an F0 mosaic mutant by using the CRISPR–Cas9 system [3], but also a homozygous F2 mutant in cultures of this species. Our results suggest that sea urchins may become more attractive model organisms for biological research with the introduction of genetic methods and the abundant knowledge of these organisms accumulated by previous researchers.

The introduction of molecular genetics into biological studies beginning in the 20th century has dramatically advanced our knowledge of model organisms because these methods enable us to understand gene function very precisely. On the other hand, non-model organisms, in which it may not have been possible to introduce these techniques for reasons such as long breeding cycles, have been less attractive to biologists. Nevertheless, the answers to a number of intriguing and important biological questions remain to be obtained in model species. Sea urchins were long considered important model organisms in cell and developmental biology [1,2], but they are no longer recognized as such because their long breeding cycle, which may take 1 or 2 years [4], has prevented the introduction of genetic techniques in these echinoderms. Therefore, despite the success of gene editing using the CRISPR–Cas9 system [5–7], it is realistic that we might obtain only F0 mutants, sometimes presenting mosaic genomes in individual cells.