

both in terms of taxonomic diversity and geographic extent, could be explained at least in part by the rapid training of predators through the use of multisensory aposematism. While birds are typically implicated as the predator driving the evolution of shared color patterns in mimicry systems [7], there is little evidence that birds regularly attack velvet ants. Instead, diurnal lizards may be the key selective agent in the evolution of mimicry in these animals [2]. This unique combination of predator and prey, and the fact that both can easily be kept in controlled laboratory settings, could allow for more experimentation than would be possible in systems driven by avian predation.

Yet another interesting feature of this system is that male velvet ants are relatively harmless compared to their female counterparts. However, the males often still have aposematic coloration but do not always resemble or even participate in the same mimicry ring as their conspecific females. This phenomenon has been termed dual sex-limited mimicry [8], which is a form of automimicry. While relatively few velvet ant sex associations have been made (for example, fewer than 30% of *Dasymutilla* species are known from both sexes), the use of molecular techniques to improve taxonomy [9] will undoubtedly reveal additional synonymies and sex associations and will facilitate studies of automimicry in male velvet ants.

Finally, velvet ants exhibit a wide array of mimetic fidelity, with many species within a given mimicry ring being nearly indistinguishable and others being only vaguely similar [4], a phenomenon known as imperfect mimicry. Because the models (mimicry rings) and the mimics (individual members of each mimicry ring) are now relatively well defined in this vast velvet ant mimicry complex, hypotheses about mimetic fidelity and imperfect mimicry can be rigorously evaluated. For example, analyses of imperfect mimicry in the *Dasymutilla* mimetic system refuted the body size hypothesis (a.k.a. relaxed selection hypothesis [4]) that found support in hoverflies [10]. Instead, preliminary support in *Dasymutilla* was found for the community diversity hypothesis, which posits lower overall mimetic fidelity in geographic areas that harbor a high diversity of models [4]. We

expect that other novel hypotheses will be generated and tested as more pieces of the velvet ant mimicry complex fall into place.

#### SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures, two tables and four figures can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.06.053>.

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## Correspondence

# Whistled Turkish alters language asymmetries

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Whistled languages represent an experiment of nature to test the widely accepted view that language comprehension is to some extent governed by the left hemisphere in a rather input-invariant manner [1]. Indeed, left-hemisphere superiority has been reported for atonal and tonal languages, click consonants, writing and sign languages [2–5]. The right hemisphere is specialized to encode acoustic properties like spectral cues, pitch, and melodic lines and plays a role for prosodic communicative cues [6,7]. Would left hemisphere language superiority change when subjects had to encode a language that is constituted by acoustic properties for which the right hemisphere is specialized? Whistled Turkish uses the full lexical and syntactic information of vocal Turkish, and transforms this into whistles to transport complex conversations with constrained whistled articulations over long distances [8]. We tested the comprehension of vocally vs. whistled identical lexical information in native whistle-speaking people of mountainous Northeast Turkey. We discovered that whistled language comprehension relies on symmetric hemispheric contributions, associated with a decrease of left and a relative increase of right hemispheric encoding mechanisms. Our results demonstrate that a language that places high demands on right-hemisphere typical acoustical encoding creates a radical change in language asymmetries. Thus, language asymmetry patterns are in an important way shaped by the physical properties of the lexical input.

There are a number of whistled languages and they all are spoken by small groups of peoples, such as some villagers in Turkey [8,9]. Whistled Turkish uses the full lexical information and syntactic codes of Turkish, but transforms them into whistles that vary in pitch and melodic line [8]. Whistlers speak normal articulated Turkish when

communicating at short range, but switch to whistled Turkish when conversing over long distances of up to several kilometers (Supplemental movie).

While acoustic variables like pitch, timbre, and melodic line are sometimes emulated by whistled languages [8], spoken languages additionally feature phonological properties of auditory speech input at shorter time windows. Two neural streams connecting posterior and anterior cortical regions are involved in speech processing; a strongly left-lateralized dorsal stream that maps acoustic speech signals to articulatory networks and a less lateralized ventral stream that processes speech comprehension [1]. Left and right auditory cortical areas seem to be specialized to temporal information from short time windows and spectral information from longer time windows for both speech and non-speech sounds, respectively [1,6]. Thus, speech-based formant transitions would mostly fall into left hemisphere specialization, while suprasegmental prosodic information fall within the right hemisphere temporal window [7]. Examining hemispheric specialization of whistled Turkish language encoding provides a unique opportunity to test whether a language that conveys full lexical and syntactic left-hemisphere dependent information and, at the same time, uses the acoustic characteristics for which the right hemisphere is specialized, departs from the usual pattern of left-hemisphere dominance.

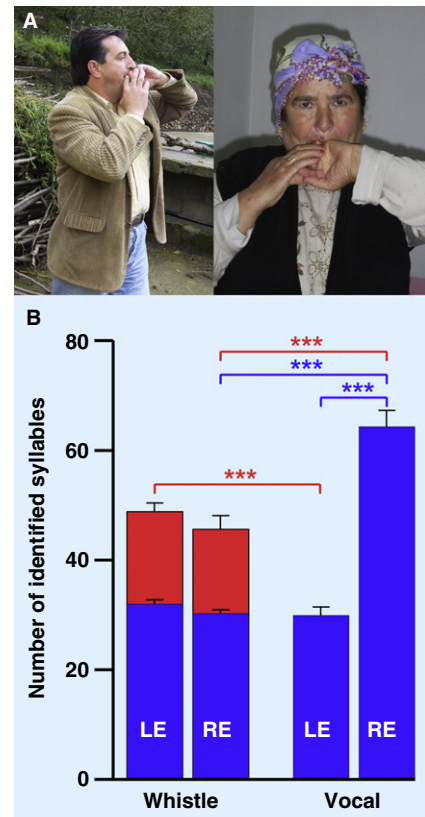
We used the dichotic listening paradigm to study language asymmetry. In a dichotic listening task, participants simultaneously hear via headphones either same (homonymic) or different syllables (dichotic condition) on left and right ears. Then they report what they perceived. Dichotic listening tests reveal that usually right ear input is perceived, which is related to left hemisphere speech sound processing. We tested 31 whistled Turkish-speaking participants with vocal and whistled syllables in a within-subject design and compared their lateralized performance between these two conditions (Supplemental information). The participant's dichotic performance was expressed as a laterality index (LI) that varies between -100 and +100 with positive values for right ear and negative values for left ear advantage processing.

We found a clear lateralization pattern for vocal dichotic listening ( $LI = 32.29 \pm 31.90$ ; SD) with significantly more syllables identified by right ( $63.87 \pm 16.64$ ) than by left ear ( $29.94 \pm 12.37$ ;  $t_{30} = 7.12$ ,  $P = 6.4 \times 10^{-8}$ ). Contrary, whistle dichotic listening evinced no lateralization ( $LI = -3.03 \pm 12.03$ ; left ear:  $32.23 \pm 5.57$ ; right ear:  $30.48 \pm 6.26$ ) ( $t_{30} = 1.23$ ,  $P = 0.23$ ). In all but two subjects, listening to whistled speech elicited reduced hemispheric dominance compared to spoken speech (Supplemental information). The number of syllables identified by the left ear was similar for vocal and whistle syllables ( $t_{30} = 0.96$ ,  $P = 0.34$ ), while right ear responses were decreased for whistles ( $t_{30} = 11.49$ ,  $P = 2.0 \times 10^{-12}$ ) (Figure 1).

Vocal dichotic syllables ( $93.81 \pm 12.49$ ) were better recognized than whistled ones ( $62.71 \pm 8.96$ ;  $t_{30} = 12.68$ ,  $P = 1.4 \times 10^{-13}$ ), although whistled syllables were still recognized well above chance level ( $52.26\% \pm 7.38$  vs.  $33.3\%$  for two out of six stimuli; Supplemental information). It is known that transformation of articulation into whistles reduces comprehension, although still remaining intelligible. Indeed, we found that fewer homonym stimuli were correctly identified during whistle ( $37.70\%$ ) than during vocal language recognition ( $75.00\%$ ;  $t_{30} = 13.22$ ,  $P = 4.77 \times 10^{-14}$ ). Again the lower response rate of the whistle homonym stimuli did not reflect a chance response bias, which would be  $16.7\%$  (one out of six syllables).

We therefore calculated an 'adjustment factor' by dividing the number of correct vocal dichotic stimuli by the number of correct whistle dichotic stimuli ( $1.52 \pm 0.25$ ). Adjusting the responses to dichotic whistle stimuli by this factor showed that the lack of asymmetry for whistle language was associated both with a decrease of right ear ( $46.26 \pm 9.49$ ;  $t_{30} = 5.75$ ,  $P = 2.83 \times 10^{-6}$ ) and an increase of left ear performance ( $48.90 \pm 8.45$ ;  $t_{30} = 5.75$ ,  $P = 2.83 \times 10^{-6}$ ).

Up to now, the language specialization of the left hemisphere was seen as being rather input-invariant. Our results require a modification of this assumption as they demonstrate that language asymmetry is shaped by the physical properties of the lexical input. The increase of right-hemisphere performance possibly results from the right-hemisphere typical acoustic properties of whistled Turkish. Formant transitions are present



**Figure 1. Brain asymmetry of whistled Turkish.** (A) Two whistle language speakers in Kuşköy, Northeastern Turkey. (B) Left ear (LE) and right ear (RE) responses for whistle and vocal dichotic syllables. Blue histograms represent the number of correctly identified syllables. Red histograms show whistle responses adjusted for comparable recognition performance between whistle and vocal dichotic listening task. Significant differences displayed in blue refer to the number of identified syllables. Significant differences displayed in red refer to the number of adjusted whistle responses. All differences were significant at  $p < 0.001^{***}$ . Variance bars are standard errors.

in whistled Turkish, but are more simply expressed as a modulated pitch, which might overall relatively reduce left-hemisphere performance [9] (Supplemental information). Overall, these processes possibly created a symmetric hemispheric performance.

One study had previously analyzed the neural correlates of a whistled language but without collecting perceptual output measures [10]. Therein, shepherds from La Gomera had to listen passively to whistled Spanish sentences and to monitor words. They demonstrated activation in the language areas of left and right superior posterior temporal

gyri and also saw less ventral stream temporal activation during whistle speech than during speech processing. Now, we also show left- and right hemispheric contributions that result in an absence of cerebral asymmetries during whistle language encoding. Thus, a natural but acoustically different language can create a radical change in the organizational dynamics of language asymmetries.

#### SUPPLEMENTAL INFORMATION

Supplemental Information containing a one figure, two tables and a movie can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.06.067>.

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# Is the expression of sense and antisense transgenes really sufficient for artificial piRNA production?

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Animals have evolved an elegant defense system against a diverse range of selfish elements such as transposons. In animal germ line cells, PIWI proteins and small RNAs associated with PIWI proteins (piRNAs) are at the heart of this defense system. piRNAs are 23–30-nt-long small RNAs that act as sequence-specific guides for PIWI proteins. PIWI proteins possess a slicer activity that is guided by piRNAs; the PIWI–piRNA complex thus silences transposon activity by cleaving transposon RNAs [1]. At present, how *de novo* piRNA production occurs against a new non-self element is largely unknown. A recent study by Itou *et al.* [2] using reporter transgenic mice concluded that the concomitant expression of sense and antisense RNA transcripts is sufficient for piRNA production. Our bioinformatic analysis using the same piRNA datasets, however, demonstrates that the introduction of the antisense reporter construct alone produces transgene-derived piRNAs, which is inconsistent with a part of the conclusions of Itou *et al.* [2].

The biogenesis of piRNAs initiates with the fragmentation of putative long, single-stranded piRNA precursors by Zucchini endoribonuclease [3,4]. The fragmented RNAs are incorporated into a subset of PIWI proteins with a specific nucleotide preference for uracil (1U) at the 5' end of the associated RNA [5]. The 3' end of the associated RNA is then trimmed by an unidentified nuclease called Trimmer. This process is called the primary processing pathway. The

PIWI–primary piRNA complexes then cleave their complementary targets across from positions 10 and 11 from the guide piRNAs [1]. The 3' RNA fragments are in turn incorporated into another subset of PIWI proteins, and again processed into mature secondary piRNAs with adenine at position 10 (10A) that precisely overlaps with 1U piRNAs by 10 nt [1]. Next, these secondary 10A piRNAs generate secondary 1U piRNAs by cleaving their complementary target RNAs. This cleavage-dependent piRNA biogenesis is called a ping-pong amplification cycle [1]. The system has been shown to be broadly conserved among animals including flies, mice, zebrafish, and silkworm.

How does *de novo* piRNA production initiate against a new invading non-self element? To solve this issue, transgenic approaches using *EGFP*-expressing transgene cassettes have been utilized in flies, mice, and silkworm [6–8]. The results of these studies clearly show that the integration of a transgene cassette into the specific, active piRNA cluster is critical for *de novo* piRNA production. The recent study by Itou *et al.* [2] published in *Current Biology*, however, appears to upend this concept. They developed an artificial piRNA production system in mice by expressing sense and antisense *EGFP* mRNAs in embryonic male germ cells during the piRNA biogenesis stage. On the basis of these experiments, they argue that concomitant expression of sense and antisense RNA transcripts is necessary and sufficient for piRNA production and subsequent piRNA-dependent gene silencing [2].

To investigate why such a discrepancy is observed between the study by Itou *et al.* [2] and previous studies [6–8], we performed bioinformatic analysis using piRNA data that were deposited in the public database by Itou *et al.* We note that we only used a part of the deposited data because the data included variable length sequences both with and without adaptor trimming (the data marked in Figure 1A were used in our analysis). We therefore focused on mapping patterns of piRNAs but not on amounts of mapped piRNA reads (normalized mapped reads are shown in Table S1 in Supplemental