

two. Ethologist Nikolaas Tinbergen emphasized the functional role of song in mate selection and territorial defense, whereas ornithologist W.H. Thorpe distinguished between songs and calls on the basis of duration and complexity, considering the longer and more complex vocalizations to be songs. More recently, cognitive scientists such as Tecumseh Fitch have focused on whether a vocal behavior is learned or innate, treating all learned vocalizations as songs and all innate vocalizations as calls, regardless of their aesthetic qualities. This ‘vocal learning’ model has become influential, in part because vocal learning species tend to be those that most frequently display proto-musical behaviors. Moreover, rhythmic entrainment has until now only been reported in vocal learning species.

Only a few animal clades are thus far known to include vocal learning species — among birds, parrots, hummingbirds, and oscine songbirds, and among mammals, humans, cetaceans, pinnipeds, elephants, and bats. Animals of these species must be exposed to representative adult songs in order to develop a typical adult song. One consequence of songs being a learned rather than an innate behavior is that a number of songbird species display geographically-based dialects. This is also the case for humpback whales, whose songs tend to be similar within a group but vary across geographic areas.

What lies ahead for zoomusicology?

Although zoomusicology has already given us many intriguing findings, the field is still in its infancy. There are more than 4000 species of birds, and a growing list of mammals, recognized as vocal learners. Each has its own pattern of song acquisition and structure, and must be studied individually to be understood. Basic pitch and rhythmic analysis constitute an obvious first approach to analyzing animal songs, and more advanced methods of structural, spectral and timbral analysis, including computational approaches originating from the burgeoning field of music information retrieval, potentially hold even greater promise.

A related question is whether animals and humans perceive musical sounds in a similar manner. Although research in this field is still in its early stages, studies on pitch, timbre, and rhythm perception in animals suggest that vocal learning species may have better auditory discrimination abilities than non-vocal learning species, and that, in certain contexts, non-human species do appear to have preferences for some musical sounds over others. Encouragingly, interest for this topic, and more generally for a comparative approach to zoomusicology, is steadily growing.

While it may not be possible to determine conclusively whether animal song is music, getting musicologists, scientists, and philosophers to join forces in zoomusicological inquiry will surely lead to a better understanding of animal song, and to a better understanding of human music as well.

Where can I find out more?

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Vectored antibody gene delivery mediates long-term contraception

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Development of non-surgical methods of long-term or permanent contraception remains a challenge. Towards this objective, we show that intramuscular injection of a replication-incompetent, recombinant adeno-associated virus (rAAV) designed to express an antibody that binds gonadotropin-releasing hormone (GnRH), a master regulator of reproduction in vertebrates, results in long-term infertility in male and female mice. Female mice are also rendered infertile through rAAV-dependent expression of an antibody that binds to the zona pellucida (ZP), a glycoprotein matrix that surrounds the egg and functions as a sperm-binding site. Many proteins known or suspected to be important for reproduction can be targeted, potentially reversibly, using this approach, which we refer to as vectored contraception (VC).

Mature GnRH is a 10-amino-acid peptide of identical sequence in most mammals, produced in the hypothalamus. GnRH is released into the median eminence, from which it diffuses into the hypophyseal portal capillary system, which carries it to the anterior pituitary, where it stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the general circulation. FSH and LH promote the formation of gametes and the production of sex steroids. Thus, loss of GnRH results in male and female infertility, and loss of other steroid-hormone-dependent traits [1]. Inhibition of GnRH is considered to be an attractive strategy in animals when the goal is to inhibit fertility and sex-steroid-dependent traits, such as aggression and territoriality. Vaccination with GnRH can inhibit

fertility, but these effects are often temporary [2].

We used mass spectrometry to determine the protein sequence of the anti-GnRH monoclonal antibody SMI41 [3] (see Supplemental Experimental Procedures and Figure S1A in the Supplemental Information). Gene synthesized heavy and light chains were shown to produce a high-affinity anti-GnRH antibody (Figure S1B) and were introduced into an AAV2/8 vector [4], generating AAV-SMI41 (Supplemental Experimental Procedures). Viral particles were administered to mice at several doses through injection of the gastrocnemius muscle (Table S1). Figure 1A,B, Figure S1C and Table S1 summarize the results of mating experiments. Females with titers <200 $\mu\text{g/ml}$ showed dose-dependent subfertility (reduced progeny numbers; Figure S1C), while females with titers >200 $\mu\text{g/ml}$ were, with several exceptions, infertile, as was one animal with a final titer of 129.7 $\mu\text{g/ml}$ (#17) (Figure 1A). The four exceptional females (#6, #12, #15, #21) had titers >200 $\mu\text{g/ml}$ at the beginning of the four-week period in which pregnancy was scored, but each experienced a significant drop in titer by the end of week four (#6, 266.2 \rightarrow 64.6 $\mu\text{g/ml}$; #12, 201.7 \rightarrow 186.0 $\mu\text{g/ml}$; #15, 219.2 \rightarrow 30.3 $\mu\text{g/ml}$; #21, 379.7 \rightarrow 45.6 $\mu\text{g/ml}$), which may explain the restoration of fertility (Figure 1A, Table S1). Males with titers >100 $\mu\text{g/ml}$ were infertile (Figure 1B). Control animals tested for fertility at 4–6 months, and 12–14 months of age were, with one exception, fertile (Figure S1D).

Ovaries of untreated females contained multiple stages of oogenesis, had few atretic follicles, and contained multiple corpora lutea, indicative of regular oocyte maturation and ovulation (Figure 1C). In contrast, ovaries from infertile anti-GnRH-expressing females contained many atretic follicles, and preovulatory follicles and corpora lutea were not observed (Figure 1D). These phenotypes, indicative of a failure to complete follicle development, resemble those of mice lacking GnRH [1].

In males GnRH-dependent release of LH is required for the production of testosterone by testicular Leydig cells. Serum testosterone levels of

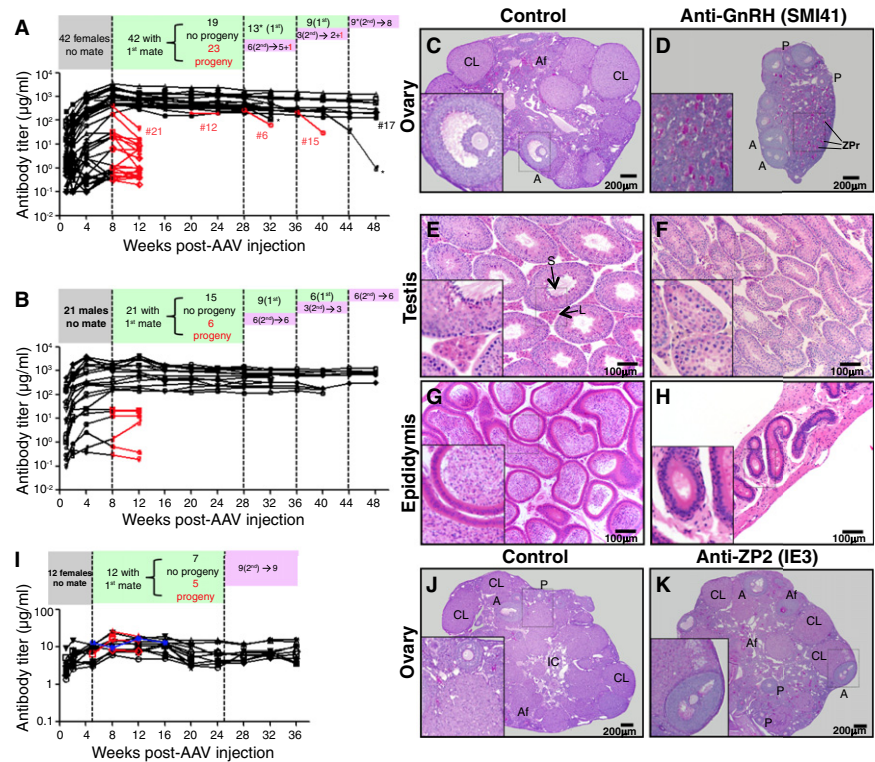


Figure 1. Vectors expression of the anti-GnRH antibody SMI41 or the anti-ZP2 antibody IE3 results in dose-dependent long-term infertility.

(A) SMI41 titers in females are plotted over time. Red line segments indicate four-week intervals during which a female became pregnant; black line segments indicate intervals during which no pregnancies occurred. Individuals are labeled as in Table S1. Vertical dashed lines indicate points at which AAV-SMI41 females were introduced to new breeding partners. The number of females maintained with either the first male mating partner (1st mate, green regions), or a second mating partner (2nd, purple rectangles), is indicated. Asterisks identify females who died. Note that some infertile females were euthanized for histology at weeks 32 and 40 (Supplemental Experimental Procedures). (B) SMI41 titers in males, characterized as in (A), but with female mating partners. (C) PAS-stained section from the ovary of control 12-month-old female. Antral follicles (A), atretic follicles (Af) and multiple corpora lutea (CL) are labeled. (D) PAS-stained section of ovary from sterile anti-GnRH-expressing 12-month-old female (#8). Primary follicles (P) and numerous remnants of the zona pellucida, associated with follicle atresia (ZPr, arrows) are indicated. (E) H&E-stained section from testis of a 14-month-old control male. Sperm in the tubule lumen (S) and large Leydig cell islands (L) between tubules are marked. (F) H&E-stained section from 14-month-old AAV-SMI41 male (#48). (G) Section through the cauda epididymis of control male from (E). (H) Section through the cauda epididymis of AAV-SMI41 male #48. (I) IE3 antibody titers of female mice injected with AAV-IE3 are plotted over time as above. The blue line indicates a female (#74) who gave birth twice. (J) PAS-stained section from the ovary of a 7-month-old control female. (K) PAS-stained section of ovary of 7-month-old AAV-IE3 female #75.

controls ranged from 0.11 ng/ml to 8.01 ng/ml ($n = 11$; mean \pm SEM, 1.65 ± 0.77). In contrast, testosterone was undetectable in infertile SMI41 males ($n = 8$). Testes of infertile males were also significantly smaller than those of age-matched controls ($n = 15$; 12.57 ± 1.25 mm, vs $n = 18$; 30.2 ± 1.159 mm; $p < 0.01$) (Figure S1E,F). All stages of spermatogenesis, including elongated spermatids, were present in testes of control mice (Figure 1E), and sperm were abundant in the

epididymis (Figure 1G). In contrast, testes of high-titer infertile males lacked late-stage spermatids (Figure 1F), and sperm were absent from the epididymis (Figure 1H), phenotypes similar to those of males lacking GnRH [1] or the β subunit of LH [5].

The ZP consists of a matrix of three or four glycoproteins (ZP1–4) synthesized by the growing oocyte, surrounding it and the early embryo. It serves as an initial binding site for sperm, an interaction required for

sperm to penetrate the ZP and fuse with the egg plasma membrane [6]. Vaccination with solubilized ZP or isolated ZP proteins can bring about infertility. Sometimes the effects are transient; alternatively, they can be long-lasting, but may be associated with reproductive tissue damage, due to the induction of antibody- and/or T-cell-mediated responses [7].

We cloned rat IE3, a monoclonal antibody that binds the amino terminus of mouse ZP2, a critical ligand for taxon-specific sperm binding [8] (Figure S2A). IE3 transiently inhibits female mouse fertility when introduced as ascites fluid through intraperitoneal injection [9]. Recombinant IE3 localized to the egg ZP *in vitro* and *in vivo* (Figure S2B). When mating was initiated 5 weeks after injection of AAV-IE3, seven out of twelve mice expressing IE3 were infertile, while five were fertile, but with reduced litter sizes compared with controls (mean \pm SEM, 3.8 ± 1.35 vs 14.7 ± 0.94 ; $p < 0.01$) (Figure 1, Figure S2C). However, over subsequent months all AAV-IE3 mice (titer range between 2.8 and 19.3 $\mu\text{g/ml}$) failed to produce progeny, with the exception of one of the initially fertile group of five, who delivered a second litter of one during month three of mating (Figure S2D). Importantly, ovaries from infertile females expressing IE3 for 6 months (Figure 1, compare panels K and J), as with those exposed to IE3 transiently [9], appeared histologically normal, and contained follicles at multiple stages of development, as well as multiple corpora lutea. This, in conjunction with the observation that comparable numbers of eggs can be superovulated from control and AAV-IE3 females (Figure S2B), suggests that vectored expression of anti-ZP antibodies can induce long-term infertility without disrupting normal follicular maturation.

SUPPLEMENTAL INFORMATION

Supplemental information, including background, discussion, hurdles that must be overcome in implementation of vectored contraception, possible strategies for bringing about reversibility, experimental procedures, two figures and one table, can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.08.002>.

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Correspondence Eurasian reed warblers compensate for virtual magnetic displacement

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Displacement studies have shown that long-distance, night-migrating songbirds are able to perform true navigation from their first spring migration onwards [1,2]. True navigation requires both a map and a compass. Whereas birds are known to have sun, star, and magnetic compasses, the nature of the map cues used has remained highly controversial. There is quite strong experimental evidence for the involvement of olfactory map cues in pigeon and seabird homing [3]. In contrast, the evidence for the use of magnetic map cues has remained weak and very little is known about the map cues used by long-distance migratory songbirds. In earlier experiments [2,4], we have shown that Eurasian reed warblers physically displaced 1,000 km eastward from Rybachy to Zvenigorod (Figure 1) re-orient towards their breeding destinations by changing their orientation in Emlen funnels from the NE to the NW. We have also previously shown that this re-orientation cannot be explained by a ‘jetlag effect’ [5]. We have now used this model system to show that Eurasian reed warblers use geomagnetic map cues to determine their position.

We performed orientation experiments in Emlen funnels with birds captured during spring migration in Rybachy (55° 09' N, 20° 52' E; Figure 1). The birds were housed outdoors in wood and cloth-net cages, which provided the birds with a clear view of the sky and the surroundings (see Figure S1 in the Supplemental Information). First,