# Birdsong: not all contest but also cooperation?

Riebel, K.; Langmore, N.E.

are several possible ways in which the *C. crescentus* OPG may contribute to cell-envelope health. One possibility is that the production and modification of the OPG modulates the expression, selectivity, or transport ability of porins, the primary entryways for many antibiotics, as previously suggested[5]. This, however, would be inconsistent with the observation that EstG is required to survive a non-antibiotic cell wall stress condition, namely the overexpression of a toxic cell division protein fragment used in the initial screen.

Though speculative, a second plausible scenario is that the balance between modified and unmodified OPG serves to calibrate periplasmic osmolarity by modulating the Donnan potential, and thus potentially influencing cytoplasmic turgor, in response to environmental changes. Accumulation of a periplasmic solute (especially if paired with porin reduction or closing) may reduce water influx into the cytoplasm, resulting in reduction in turgor and thus mitigation of cell-wall stresses (which are likely exacerbated by high internal turgor). This model would be consistent with the observed rescue of Δ*estG* defects by growth in high osmolarity conditions, which would reduce water flux into the cytoplasm. The authors suggest that EstG might modify the charge of the OPG, which would be in line with a Donnan-potential-dependent effect. The periplasmic-osmolarity model could also be consistent with the unmodified form being detrimental — perhaps a specific ratio between a charged and an uncharged form (rather than the mere existence of either form) is the optimal response to cell-envelope problems. Alternatively, modified OPG might indirectly facilitate the activity of an envelope-stress response that is required for survival of cell-wall stress. Whatever the exact mechanistic details may be, the *C. crescentus* OPG provides some intriguing leads, and these observations once more illustrate that there is much left to learn about how bacteria respond to stressful environments. Since clinical therapy induces stressful environments for bacteria, and bacterial resistance to therapy can rely heavily on stress-response systems, such knowledge might lead to the development of novel antibiotics and their adjuvants in the future.

## DECLARATION OF INTERESTS

The author declares no competing interests.

## REFERENCES

1. Kennedy, E.P. (1982). Osmotic regulation and the biosynthesis of membrane-derived oligosaccharides in Escherichia coli. Proc. Natl. Acad. Sci. USA *79*, 1092–1095.

2. Bontemps-Gallo, S., Bohin, J.P., and Lacroix, J.M. (2017). Osmoregulated periplasmic glucans. EcoSal Plus 7, https://doi.org/10.1128/ecosalplus.ESP-0001-2017.

3. Bontemps-Gallo, S., and Lacroix, J.M. (2015). New insights into the biological role of the osmoregulated periplasmic glucans in pathogenic and symbiotic bacteria. Environ. Microbiol. Rep. *7*, 690–697.

4. Bohin, J.P. (2000). Osmoregulated periplasmic glucans in Proteobacteria. FEMS Microbiol. Lett. *186*, 11–19.

5. Fiedler, W., and Rotering, H. (1988). Properties of *Escherichia coli* mutants lacking membrane-derived oligosaccharides. J. Biol. Chem. *263*, 14684–14689.

6. Daitch, A.K., Orsburn, B.C., Chen, Z., Alvarez, L., Eberhard, C.D., Sundararajan, K., Zeinert, R., Kreitler, D.F., Jakoncic, J., Chien, P., *et al*. (2023). EstG is a novel esterase required for cell envelope integrity in *Caulobacter*. Curr. Biol. *33*, 228–240.

7. Mahasenan, K.V., Batuecas, M.T., De Benedetti, S., Kim, C., Rana, N., Lee, M., Hesek, D., Fisher, J.F., Sanz-Aparicio, J., Hermoso, J.A., and Mobashery, S. (2020). Catalytic cycle of glycoside hydrolase BglX from *Pseudomonas aeruginosa* and its implications for biofilm formation. ACS Chem. Biol. *15*, 189–196.

8. Alegun, O., Pandeya, A., Cui, J., Ojo, I., and Wei, Y. (2021). Donnan potential across the outer membrane of Gram-negative bacteria and its effect on the permeability of antibiotics. Antibiotics *10*, 701.

# Birdsong: Not all contest but also cooperation?

**Katharina Riebel[1,*] and Naomi E. Langmore[2]**
[1]Institute of Biology, Leiden University, Leiden, The Netherlands
[2]Research School of Biology, Australian National University, Acton, Australia
*Correspondence: k.riebel@biology.leidenuniv.nl
https://doi.org/10.1016/j.cub.2022.12.015

**Birdsong generally functions to defend territories from same-sex competitors and to attract mates. Wild zebra finch males now are shown to sing prolifically outside the breeding season and without defending territories, suggesting potential social functions for birdsong beyond competition.**

Through the ages, melodious birdsong has piqued the aesthetic senses of artists, writers researchers and lay people alike[1]. Darwin's ideas about sexual selection by mate choice were likewise inspired by birds' dazzling visual and acoustic ornaments. When these ideas were tested in earnest during the last century, little of romanticism was left: birdsong turned out to mediate intense competition over territories and mates. This view has become increasingly nuanced — song not only attracts and defends mates, it also stimulates reproductive activity, aids maintenance

**Figure 1. Zebra finches in the wild.**
A group of wild zebra finches at Gap Hills (NSW, Australia). The photograph shows several birds of both sexes, with two adult males (red cheek patches) perching on the same branch in close vicinity (photo: Marc Naguib).

of pair bonds or attracts extra-pair matings. These additional functions still align with the classic view of birdsong as ornament (to attract mates) and armament (to repel same-sex competitors). However, more comprehensive research on song usage in other seasons and contexts, often aided by better tracking and semi-automatic recording methods, increasingly documents song in contexts outside this framework[2]. This is exemplified by a new study in this issue of *Current Biology* by Hugo Loning, Marc Naguib and colleagues[3] showing that male zebra finches (*Taeniopygia guttata*; Figure 1) sing not only during the breeding season, but all year-round and in a variety of social contexts. Although zebra finches do not defend territories, but instead live in loose colonies, Loning and colleagues[3] show that males sing prolifically also outside the breeding season, both alone and in company and mostly when with their long-term mate. This is inconsistent with the traditionally assumed functions of mate attraction and territory defense, suggesting that a broader framework of natural selection might be needed for understanding the evolution and current functions of birdsong.

Zebra finches are a common songbird of Australia and the lesser Sunda Islands of Indonesia. They are colonial breeders adapted to an arid climate, breeding opportunistically following unpredictable rainfall and they maintain stable pairbonds throughout the year. Zebra finches are one of the most intensively studied bird species[4] and an important model in understanding the function of song and its (neural) development. Zebra finch song is sexually dimorphic; both sexes produce calls but only males produce complex song. As in other songbirds, song is learned early in life from conspecifics. Zebra finches sing in two modes: 'directed songs', in close vicinity and orientated toward a conspecific as part of a courtship display, and 'undirected songs', which are not directed towards any other individual, and can occur when the bird is either alone or in company[5,6].

To some extent, zebra finch song conforms to the traditional functions of mate attraction, mate stimulation and mate defence. First, directed song is an essential component of courtship, because females do not solicit copulations when males do not sing[7]. Second, in captive zebra finches, males with high rates of undirected songs obtained more extra-pair copulations[8]. Similarly, in the wild, undirected song rates correlated with rates of extra-pair directed song and increased immediately after experimental mate removal[9]. In experimental studies, undirected song also attracted females and predicted their choices of live males[10]. And as shown by Loning and colleagues[3], males sing at higher rates during the egg-laying than during incubation and nestling stages, suggesting that song may play a role in guarding or courting the fertile mate.

However, Loning and colleagues[3] also observed song in a much wider range of contexts. They used automatic continuous recording devices to confirm and quantify earlier but mostly ignored observations[6,9] that males consistently produce song in non-breeding contexts. They propose that these songs serve a cooperative role, helping to synchronise initiation of breeding or to coordinate a pair's foraging or dispersal movements. These findings are consistent with studies of vocal communication in other species that breed in aseasonal environments. In unpredictable climates, birds often show a quick breeding response to favourable conditions and song may be used to synchronise reproductive physiology, by initiating the neuroendocrinological processes that lead to reproduction[11]. For example, in female white-crowned sparrows (*Zonotrichia albicollis*), male song triggers the release of luteinizing hormone within an hour[11]. The finding of Loning and colleagues[3] of prolific singing outside the breeding season is also consistent with studies on communication in other non-territorial, group-living species. Flocking presents challenges in terms of coordinating group movements and social interactions, and vocalisations can mediate these interactions. For example, group-living parrots typically use contact calls to coordinate group movements and soft calls to mediate interactions at foraging patches[12]. Similarly, in some flocking songbirds, such as European starlings (*Sturnus vulgaris*), songs are produced year-round and function in a variety of social contexts, such as mediating spacing at roosts and long distance interactions between males[13].

In line with the idea that songs have social functions outside the breeding season and pairbond, Loning and colleagues[3] observed that playbacks attracted males without triggering agonistic interactions. These observations place previous laboratory findings in a new perspective. In captive studies, males do not necessarily

increase singing when introduced to potential competitors[14], and males, like females, are willing to work in operant tests to trigger song playback and more so for songs they heard early in life[15]. In combination with the observation of Loning and colleagues[3] that song attracts males in the wild, these findings suggest several potential song functions, such as aiding decisions about with whom to socialise, or where to settle, or in facilitating kin recognition. While it is known that exposure to song stimulates or synchronises breeding in females, this effect is normally studied in pairs[16]. It is possible that, in wild flocks, male song may also stimulate reproductive behaviour in other males. Interestingly, Loning and colleagues[3] reveal that song attracts both sexes outside the pairbond and breeding context, suggesting that song might function as a social attractant, a function that has rarely been described in songbirds but might explain year-round song in colonial species, such as starlings[13], or in species whose territories break down in the non-breeding season, such as superb fairy-wrens (*Malurus cyaneus*)[17]. However, if song is a year-long social attractant, the extreme sexual dimorphism in singing behaviour in zebra finches is puzzling. In many closely related species, both sexes sing[18], which makes the absence of song in female zebra finches even more intriguing and challenges us to conduct more comparative research to identify the ecological correlates of female song[2].

The findings by Loning and colleagues[3] show how much there is still to learn about song usage in the wild even in one of the most intensively researched song bird species[4]. Traditionally, song was thought to have evolved through sexual selection acting on males to facilitate competition for mates and territories and most research has focussed on these contexts. However, a paradigm shift is afoot because new research shows song production by females is common[19] and that song might serve a wider variety of competitive, cooperative and anti-predator functions[2,20]. Given these broad contexts and functions of song, it seems more likely that song has evolved through the processes of both natural selection and sexual selection. In the context of competitive use of song, there has been much recent debate about the extent to which birdsong has evolved through the broader process of social selection, rather than exclusively through the narrow process of sexual selection. Social selection encompasses all selection resulting from social competition for any resources, including both sexual and non-sexual contexts. It would thus encompass song that is produced by both males and females to defend resources such as feeding sites and non-breeding territories, as well as more familiar roles such as defence of a mate or a breeding territory. However, the theories of neither social selection nor sexual selection include cooperative functions of birdsong, such as breeding synchronisation, maintenance of pair or group cohesion or coordination of care of young. Nor do they explain song production in anti-predator contexts. Song production in these contexts is likely to have evolved through natural selection by enhancing survival and reproductive success. The study by Loning and colleagues[3] should inspire us to broaden our focus to singing outside breeding seasons and to contemplate the possibility that there might be additional functions to song, including social functions such as maintaining group cohesion. As researchers we should open our eyes — and ears— to these possibilities.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## REFERENCES

1. Doolittle, E. (2022). Birdsong and music. Curr. Biol. *32*, R1064–R1066.
2. Riebel, K., Odom, K.J., Langmore, N.E., and Hall, M.L. (2019). New insights from female bird song: towards an integrated approach to studying male and female communication roles. Biol. Lett. *15*, 20190059.
3. Loning, H., Verkade, L., Griffith, S.C., and Naguib, M. (2023). The social role of song in wild zebra finches. Curr. Biol. *33*, 372–380.
4. Griffith, S.C., Ton, R., Hurley, L.L., McDiarmid, C.S., and Pacheco-Fuentes, H. (2021). The ecology of the zebra finch makes it a great laboratory model but an outlier amongst passerine birds. Birds *2*, 60–76.
5. Caryl, P.G. (1981). The relationship between the motivation of directed and undirected song in the zebra finch. Z. Tierpsychol *57*, 37.
6. Dunn, A.M., and Zann, R.A. (1996). Undirected song encourages the breeding female zebra finch to remain in the nest. Ethology *102*, 540–548.
7. Hauber, M.E., Campbell, D.L.M., and Woolley, S.M.N. (2010). The functional role and female perception of male song in zebra finches. Emu *110*, 209–218.
8. Houtman, A.M. (1992). Female zebra finches choose extra-pair copulations with genetically attractive males. Proc. R. Soc. Lond. B *249*, 3–6.
9. Dunn, A.M., and Zann, R.A. (1996). Undirected song in wild zebra finch flocks: contexts and effects of mate removal. Ethology *10*, 529–539.
10. Holveck, M., and Riebel, K. (2007). Preferred songs predict preferred males: consistency and repeatability of zebra finch females across three test contexts. Anim. Behav. *74*, 297–309.
11. Hall, M.L. (2009). A review of vocal duetting in birds. Adv. Stud. Behav. *40*, 67–121.
12. Bradbury, J.W., and Balsby, T.J.S. (2016). The functions of vocal learning in parrots. Behav. Ecol. Sociobiol. *70*, 293–312.
13. De Groof, G., Poirier, C., George, I., Hausberger, M., and Van der Linden, A. (2013). Functional changes between seasons in the male songbird auditory forebrain. Front. Behav. Neurosci. *7*, 196.
14. Lilie, N.D., Riyahi, S., Kalinowski, A., Salazar, S.M., Kaiser, S., Schmoll, T., and Korsten, P. (2022). Male social niche conformance? Effects of manipulated opportunity for extra-pair mating on behavior and hormones of male zebra finches. Hormones Behav. *146*, 105243.
15. Riebel, K., Smallegange, I.M., Terpstra, N.J., and Bolhuis, J.J. (2002). Sexual equality in zebra finch song preference: evidence for a dissociation between song recognition and production learning. Proc. Biol. Sci. *269*, 729–733.
16. Waas, J.R., Colgan, P.W., and Boag, P.T. (2005). Playback of colony sound alters the breeding schedule and clutch size in zebra finch (*Taeniopygia guttata*) colonies. Proc. Biol. Sci. *272*, 383–388.
17. Cooney, R., and Cockburn, A. (1995). Territorial defense is the major function of female song in the sperb fairy-wren, *Malurus cyaneus*. Anim. Behav. *49*, 1635–1647.
18. Soma, M., and Garamszegi, L.Z. (2015). Evolution of courtship display in Estrildid finches: dance in relation to female song and plumage ornamentation. Front. Ecol. Evol. *3*, 4.
19. Odom, K.J., Hall, M.L., Riebel, K., Omland, K.E., and Langmore, N.E. (2014). Female song is widespread and ancestral in songbirds. Nat. Commun. *5*, 3379.
20. Tobias, J.A., Montgomerie, R., and Lyon, B.E. (2012). The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. Philos. Trans. R. Soc. B *367*, 2274–2293.