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Variation in host response to brood parasitism reflects evolutionary differences and not phenotypic plasticity

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Although adaptive traits are commonly assumed to decline when the selection pressures favouring them become relaxed, behavioural adaptations against brood parasitism often persist for long periods in isolation from brood parasites. In cases where declines have been observed, it has been unclear whether the lower expression in parasite-free populations represents genetic change or is due to phenotypic plasticity in trait expression. We experimentally tested two host species of brood-parasitic brown-headed cowbirds, *Molothrus ater*, for egg rejection behaviour in populations (1) that have bred outside the parasite's range for up to 8000 years, (2) that have been in long-term contact with cowbirds or (3) that are within the cowbird's range but are 'cowbird-naïve' because cowbirds do not breed locally. American robins, *Turdus migratorius*, and grey catbirds, *Dumetella carolinensis*, breeding in allopatry from cowbirds showed lower responsiveness to cowbird eggs than conspecifics breeding in sympatry with cowbirds. Responses of robins and catbirds in cowbird-naïve populations, which have had little or no exposure to cowbirds, were identical to those of conspecifics breeding in the presence of cowbirds, indicating that exposure to cowbirds is not necessary for the full expression of rejection and that this defence shows little or no phenotypic plasticity. Therefore, we conclude that the decline in rejection behaviour of allopatric robins and catbirds reflects genetic change and is not due to phenotypic plasticity.

Geographical variation in the structure and behaviour of organisms is typically presumed to reflect variation in the strength and direction of natural selection for adaptive traits (Endler, 1986; Foster & Endler, 1999). In general, the expression of an adaptive trait is expected to be most pronounced where selection is strongest and to decline as selection becomes relaxed, because of the costs associated with its maintenance (Rhoads, 1979). Studies of geographical variation in adaptive traits have provided many insights about evolutionary processes in nature, and some of the most important insights have come from studies that failed to find the predicted correlation between the expression of traits and the strength of selection pressures presumed to favour them (Byers, 1997; Foster & Endler, 1999; Thompson, 1999).

Brood-parasitic birds reduce the fitness of their hosts and select for host defences against parasitism, which in turn select for the evolution of counter-strategies by parasites (Davies, 2000; Rothstein, 1990). The most effective and widespread host defence is ejection behaviour, in which hosts remove parasitic eggs from the nest (Peer & Sealy, 2004b; Rothstein, 1975a). Brood parasite–host systems are ideal for studying evolutionary processes because adaptations against parasitism are rarely adaptive in any other context (Rothstein, 1990), making it easy to generate testable predictions about the extent to which hosts show defences based on the strength of selection.

Despite the clear predicted relationship between host defences and selection pressure due to brood parasitism, observed levels of defences among populations of the same host species are often discordant with their respective levels of brood parasitism. For example, hosts that currently breed in isolation from brood parasites but that are descended from lineages that previously evolved defences in the presence of parasites retain at least some egg recognition and rejection of foreign eggs placed in their nests (Briskie, Sealy, & Hobson, 1992; Peer & Sealy, 2004a; Peer, Rothstein, Delaney, & Fleischer, 2007; Rothstein, 2001), in some cases for millions of years (Peer, Kuehn, Rothstein, & Fleischer, 2011; Peer, McIntosh, Kuehn, Rothstein, & Fleischer, 2011). The evolutionary persistence of rejection behaviour in such populations suggests that this trait imposes a minimal cost of maintenance in the absence of brood parasitism (Peer et al., 2007; Rothstein, 2001), presumably because it lies dormant as a 'relic behaviour' (Coss, 1993; Rothstein, 2001; but see Peer & Bollinger, 1997) in the absence of the stimuli that elicited the response (e.g. brood parasites and their eggs). Conditionally expressed behavioural adaptations are thus expected to deteriorate more slowly than, for example, morphological traits

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that are always expressed. As an example, African village weaverbirds, *Ploceus cucullatus*, introduced to Hispaniola from Africa just 200 years ago have retained egg recognition (Lahti, 2006) but show pronounced changes in the colour and spotting of their eggs (Lahti, 2005), both of which are believed to have been adaptations against cuckoo parasitism in their native range.

Although rejection behaviour can be retained through long periods of relaxed selection, its expression has also been found to decline in some host populations that are only locally isolated from parasites (Davies & Brooke, 1989a; Lindholm, 2000), as well as in populations that have bred apart from parasites for long periods (Briskie et al., 1992; Cruz & Wiley, 1989). In these cases it is often difficult to determine whether the lower rejection rates of parasite-free populations reflect evolutionary (i.e. genetic) differences, or are instead due to phenotypic plasticity in trait expression. The latter may occur if egg recognition is elicited when hosts detect adult parasites in their breeding territories (Brooke, Davies, & Noble, 1998).

In the present study, we investigated geographical variation in the rejection behaviour of American robins, *Turdus migratorius* (hereafter 'robins') and grey catbirds, *Dumetella carolinensis* (hereafter 'catbirds'). Both species show nearly 100% rejection of nonmimetic eggs in sympatry with parasitic brown-headed cowbirds, *Molothrus ater* (hereafter 'cowbirds') in North America (Briskie et al., 1992; Rothstein, 1975a, 1982) but reduced responsiveness in populations that breed outside of the range of cowbirds. Briskie et al. (1992) showed that robins at Churchill, Manitoba, Canada, approximately 600 km beyond the northern limit of the cowbird's range, rejected cowbird eggs at significantly lower rates than conspecifics breeding in the presence of cowbirds at Delta Marsh, Manitoba (67% versus 100%, respectively). Catbirds breeding on Bermuda, where cowbirds are absent and have probably never bred, showed rejection rates that were slightly, but not significantly, lower than those of catbird populations breeding in sympatry with cowbirds on mainland North America (82.6% versus 94.5%, respectively; Rothstein, 2001). Rejection was also found to occur more slowly in the allopatric catbird population, with a significantly lower proportion of Bermuda catbirds than mainland catbirds rejecting within 1 day after the addition of a foreign egg (36.4% versus 75.9%; Rothstein, 2001).

Here we report results from new experiments on two populations of robins breeding in long-term isolation from cowbirds in central Alaska, U.S.A., where rates of cowbird egg rejection are similar to those of allopatric robins in northern Manitoba (Briskie et al., 1992). Our results, in combination with previously reported results from experiments on robins and catbirds (Rothstein, 1975a, 1982, 2001), provide further evidence for the decline of rejection behaviour in both species during prolonged isolation from cowbirds. To address whether the lower responsiveness of robins and catbirds in allopatric populations is due to phenotypic plasticity, we carried out additional experiments on both species at a 'cowbird-naïve' site within the breeding range of cowbirds in eastern Idaho, U.S.A., but where cowbirds are almost completely absent. Individuals breeding in these naïve host populations have had little to no exposure to cowbirds, but are presumed to be genetically similar to nearby populations (within 50 km) where cowbirds are abundant (see below). Thus, if the rejection responses of either species are phenotypically plastic and dependent on exposure to cowbirds, rejection should be lower in the cowbird-naïve population than in nearby populations exposed to cowbirds.

METHODS

We added dummy eggs made from plaster of Paris and painted with latex or acrylic paints and coated with a clear acrylic sealer, which are effective mimics of real eggs (see Rothstein, 1975a), to the nests of robins and catbirds to test for egg recognition in populations where these species breed (1) in sympatry with cowbirds ('sympatric' populations), (2) in long-term allopatry from cowbirds ('allopatric' populations) and (3) within the cowbird's range, but where cowbirds were very rare and where no instances of parasitism of any species were detected ('naïve' populations).

Study Areas

Allopatric populations

We tested robins breeding at two sites in central Alaska, U.S.A.; one in the vicinity of Fairbanks (64°50′N, 147°42′W) in 2001–2003 and 2007, and a second, approximately 500 km to the southwest, at Innoko National Wildlife Refuge in the areas surrounding Camp Lake (63°38′N, 158°01′W) in 2002. The northern limit of the cowbird's breeding range is approximately 1500 km from Fairbanks and 2000 km from Innoko NWR, and may have been even further south and east of our Alaska study sites prior to the settlement of North America by Europeans (Kessel & Gibson, 1978; Lowther, 1993). Catbirds were tested from 1971 to 1973 in Bermuda, where cowbirds are absent and have probably never bred (Rothstein, 2001), and some of these data have been reported previously (Rothstein, 2001). That our data were collected several decades apart should not affect the interpretation of the results (see also Peer & Rothstein, 2010). For example, it is possible that catbird rejection frequencies in allopatry on Bermuda have further declined, but this should have no effect on the overall patterns observed (see below).

Sympatric populations

Robins were tested from 1966 to 1970 in Connecticut (New Haven and Fairfield Counties) and Michigan (Cheboygan and Emmet Counties), U.S.A., while catbirds were tested from 1966 to 1972 in the same locations, as well as in Nebraska (Douglas and Dodge Counties) and Maryland (Anne Arundel County), U.S.A., and in Manitoba (at Delta Marsh). Cowbirds were present at each of these study sites, and other species (which do not reject cowbird eggs) were parasitized at moderate rates (Rothstein, 1975a). The results from experiments on robins and catbirds in sympatric populations have been previously published (Rothstein, 1975a, 1982, 2001).

Naïve population

In 2005 and 2006 we tested robins and catbirds in riparian and adjacent habitats near the junction of Kelly Creek and Moose Creek (46°43′N, 115°05′W), in the Clearwater National Forest (hereafter 'CNF') of eastern Idaho, U.S.A. This 526 000 ha tract of mostly forested, rugged mountains has limited road access and very few cowbirds, as is the case for other wild areas in Idaho (e.g. Wright, 1999; Young & Hutto, 1999). To minimize the possibility of testing the same individuals in subsequent years, we tested robins and catbirds at the main site ('Lower Kelly Creek') in 2005 and at another site approximately 2.5 km upstream in 2006 ('Upper Kelly Creek'). Cowbirds were virtually absent from the site during the entire 2005 field season, with only a single detection involving one (male) cowbird flying over the site and vocalizing without stopping. In 2006, one to three male cowbirds and three to five female cowbirds were observed on the Lower Kelly Creek site at various times. In the afternoons these birds associated with a livestock corral where outfitters intermittently kept packhorses, but they dispersed throughout the lower site in the mornings and could be heard giving perched songs, flight whistles and female chatter calls on the site during 2006, although we never observed any direct interactions between adult cowbirds and any host species. Cowbirds were only rarely observed in 2006 at the Upper Kelly Creek site where nests were tested in that year.