ISSN 2079-0864, Biology Bulletin Reviews, 2025, Vol. 15, No. 3, pp. 377–384. © Pleiades Publishing, Ltd., 2025.

Breeding Success of Interspecies Hybrids: Reduced Fertility in a Hybrid Magpie Population (*Pica pica* × *Pica serica*, Aves)

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Received December 26, 2023; revised April 11, 2024; accepted August 8, 2024

Abstract—Data on the breeding success of hybrids, obtained by means of direct observations in nature, are key to understanding reproductive barriers between species and predicting the fate of hybrid populations. Limited hybridization occurs in an emerging secondary contact zone between the Eurasian magpie *Pica pica* and Oriental magpie *Pica serica*. This was identified during our recent study of single nucleotide polymorphisms of nuclear DNA. Here we present comparative analysis of the composition of 119 breeding pairs and content of 89 nests, found in the zone of recent contact between *P. pica leucoptera* and *P. serica jankowskii* in Northeastern Mongolia and Eastern Transbaikalia, as well as in relatively pure populations in adjacent regions. In the Mongolian hybrid population we recorded higher proportions of completely non-viable clutches, as well as partially viable clutches, as compared to those of pure *P. p. leucoptera* or *P. s. jankowskii* populations. Hybridization seems less intensive in Eastern Transbaikalia, where the proportion of completely non-viable clutches, as well as the proportion of partly dead clutches, is much lower than in Mongolia. Several possible mechanisms of genetic incompatibilities and the breaking of the postzygotic isolation are discussed. The future fate of this hybrid contact zone is considered, taking into account selection against hybridization and possible reinforcement of prezygotic isolation, including differences in species-specific vocalizations. A mosaic hybrid zone with features of a “tension hybrid zone” may develop here. This unique observation of an emerging zone of contact and hybridization of two young magpie species deserves ongoing monitoring and genomic studies.

DOI: 10.1134/S2079086425700057

INTRODUCTION

Hybridization often occurs when populations or closely related species come into secondary contact after a sufficiently long period of geographic isolation. The outcome is difficult to predict, and depends on the “species’ isolating mechanisms” (Mayr, 1963), currently termed “reproductive barriers” (Mallet, 2005). The Biological Species Concept, as the basis of Synthetic Theory of Evolution, recognizes prezygotic and postzygotic reproductive barriers. The strength of these barriers depends mainly on the level of genetic divergence between the contacting populations. In the case of broken or imperfect prezygotic barriers, the success of hybridization may vary from a complete lack of any offspring due to genomic incompatibility, to a degree of success in crossing that involves introgression and/or fusion of populations, sometimes resulting in hybrid speciation. These processes taking place in the contact zones are directly connected to the issue of speciation and maintenance of species integrity.

Secondary contact is usually evaluated by the phenotypic or genotypic composition of populations and other indirect indicators. Direct, quantitative evaluations of breeding success in hybrid populations are rare due to the difficulty of making such observations in most animals. Exceptions are probably limited to insects, amphibians and birds (especially open nesting species). Magpies of the genus *Pica* (Linnaeus, 1758) provide a good opportunity in this regard. Recent secondary contact between two closely related but morphologically and genetically diverged species of magpies has been identified by our earlier integrative analysis (Kryukov et al., 2022).

Magpie *P. pica* sensu lato is widely distributed across the Holarctic, from Iberia to Kamchatka and North America. Previously, the single species contained up to 13 subspecies, including two in the Nearctic (Madge and Burn, 1999). The current taxonomy accepts five species in the Palearctic: *P. pica*, *P. mauritanica*, *P. asirensis*, *P. bottanensis* and *P. serica* (Madge et al., 2020; Gill et al., 2021). Except for the latter two, the ranges of these species are allopatric. An interesting case is observed in the relationship between *P. pica leucoptera* and *P. serica jankowskii.* These subspecies are separated by a gap in Eastern Transbaikalia and Northeastern Mongolia, which is gradually thinning and is accompanied by interspecies hybridization (Kryukov et al., 2017, 2022; Goroshko et al., 2018). The existence of that rather wide gap between species of magpies in Eastern Transbaikalia and the west of Amur Region was reported some time ago (Stegman, 1932; Rustamov, 1954; etc.). However, maps in several other papers and reviews inexplicably depict a continuous magpie range in this region (Goodwin, 1986; Zhang et al., 2012), despite no particular observations having been conducted in this area. The rather rapid westward spread of *P. s. jankowskii* along the Trans-Siberian Railway, roads and river valleys in the Upper Amur River basin was revealed only in the course of our field work (Goroshko, 2018; Goroshko et al., 2018). Meanwhile, *P. p. leucoptera* has spread eastward, albeit at a slower pace. The two species differ in coloration and size, although they are not always easily distinguishable in the field. They can be better discriminated by vocalization. Specifically, the chattering alarm calls of the two species differ, composed of fused and fast repeating elements in the western species and distinct, slow elements in the eastern.

Our field observations revealed two parts of the emerging contact zone between *P. p. leucoptera* and *P. s. jankowskii*: one in the basin of the Kherlen River in Northeastern Mongolia near the border with China, and the other in the Argun River basin in Eastern Transbaikalia, Russia also along the Chinese border. We have observed the meeting of the two species here over the course of our long-term work. Magpie population densities are very low at both sites. Nesting ranges of pure populations of the two species are close, but not completely contiguous, with a gap of 50–100 km. Notably, almost annually and sometimes massive post-breeding movements of *P. s. jankowskii* deep into the nesting range of *P. p. leucoptera* take place at both sites. This often results in a period of extended stay by *P. s. jankowskii,* at least to the winter period. When this occurs, the two species occur in mixed flocks, without signs of interspecies aggression. Some of these migrants may stay through the following summer, creating conditions for the formation of heterospecific pairs and even gene flow in the case of any successful interspecies crosses. The little existing information on similar invasions of *P. p. leucoptera* deep into the range of *P. s. jankowskii* suggests that these are of much smaller scale. Our observations and analysis of nuclear SNPs indicate that a degree of interspecies hybridization takes place at both Mongolia and Eastern Transbaikalia. Hybridization is much more intensive in the Mongolian Kherlen River basin, where there is a significant number of hybrid individuals coexisting alongside pure individuals, forming a hybrid swarm (Kryukov et al., 2022). We also observed a few hybrids in Eastern Transbaikalia. However, we did not find any breeding pairs of both pure species neighboring anywhere. According to genetic analysis, a relatively genetically pure population of *P. p. leucoptera* with isolated hybrids inhabits the territory of Central Transbaikalia adjacent to Mongolia, although no phenotypical hybrids were identified during field observations. Only pure *P. s. jankowskii* populations occur in Chinese territories adjacent to Mongolia and Eastern Transbaikalia.

One of the rationales for raising the taxonomic rank of the western and eastern subspecies groups of magpies to the distinct species *Pica pica* and *Pica serica*, which include subspecies *P. p. leucoptera* and *P. s. jankowskii,* respectively*,* was the measurement of a deep genetic divergence of 4% within the mitochondrial control region (CR) (Kryukov et al., 2004, 2017) and complete mitochondrial genome (Kryukov et al., 2020). Both species are estimated to have diverged ~2 million years ago based on molecular clock (0.94–2.7 MYA—Kryukov et al., 2004; 2.27 MYA—Song et al., 2018; or 1.68 MYA—Kryukov et al., 2022). When such genetically remote forms interbreed, genetic incompatibility, hybrid sterility and/or nonviability of hybrid progeny can be expected. Yet, we found successfully breeding mixed pairs, some of which have included a hybrid individual and produced viable offspring, and others which failed to breed successfully. In this paper, we will compare the breeding success of magpies within the hybrid zone to that in the adjacent pure populations.

MATERIALS AND METHODS

For the purpose of analysis, we distinguish four conditional populations of magpies: (1) the relatively pure population of *P. p. leucoptera* in the Onon River basin and adjacent territories of Central Transbaikalia, Russia (here referred to as "Central Transbaikalia"); (2) the Argun River basin of Eastern Transbaikalia, Russia (referred to as "Eastern Transbaikalia"), where *P. p. leucoptera* is present but the recent formation of a hybrid population of *P. p. leucoptera* and *P. s. jankowskii* may have begun in the south near the Chinese-Russian border; (3) the pure population of *P. s. jankowskii* near Ulan-Nur Lake (Orshun-Gol River basin, south of Dalainor Lake) in Northeastern China, lying south of Eastern Transbaikalia (referred to as "China"); and 4) the hybrid population in the valley of the Kherlen and Uldza Rivers of Northeastern Mongolia, located south of Central Transbaikalia (referred to as "Mongolia"). We analyzed the phenotypic composition of 119 breeding pairs over the course of field observations from 2015–2021. We used 8× binoculars, a 25–75× telescope, and a Nikon camera with a 150–500× lens for species identification in the field. Alarm calls were analyzed by ear in the field, and recorded on ZOOM H5 and Sound Devices 702 recorders with SGH-6 (ZOOM) and Telinga Stereo MK2 microphones, respectively, followed by spectral analysis.

We measured nesting success in relation to pair composition at 89 active magpie nests: 23 in Central Transbaikalia, 29 in Eastern Transbaikalia, 4 in China, and 33 in Mongolia. When nests contained clutches, the viability and stage of development of an embryo were checked by candling using a bright light source behind the egg, then, if necessary, by placing the eggs in warm water. In combination, these two methods allow for an unmistakable determination of the parameters under investigation in the vast majority of cases. Non-viable eggs cannot be recognized only in very fresh, unincubated clutches. In these cases, we checked such clutches again after a few days. All eggs identified as non-viable were autopsied, which confirmed their non-viability in all cases. When possible, nests were checked again, during the chick stage, but due to long distances and field schedules this was possible only for some nests. For the purpose of our study, a pair was considered fertile if it produced at least one chick or an undoubtedly fertilized egg, and infertile (sterile) if it had only non-viable eggs. Statistica v. 12 (StatSoft Inc.) was used to calculate statistical significance (χ^2) of fecundity parameters.

RESULTS

In Mongolia, of 33 breeding pairs, at least 11 were mixed: specifically, one partner was a hybrid according to coloration and/or call, or *P. s. jankowskii*, and the other partner was always *P. p. leucoptera*. Of the mixed pairs, 8 inhabited the Kherlen River valley in extreme Eastern Mongolia, no further than 30 km from the China-Mongolia border, and the remaining 3 mixed pairs were a bit further north, on the Uldza River basin in the northern territories of Mongolia adjacent to Russian Central Transbaikalia. The remaining 22 homospecific pairs were formed of *P. p. leucoptera* and inhabited the western part of the Kherlen River valley farther from China, as well as the Uldza River basin. In China we found only non-mixed pairs of *P. s. jankowskii*. In Eastern Transbaikalia, species composition of both partners was determined in 34 pairs: of these pairs, 21 were pure *P. p. leucoptera*, 11 pure *P. s. jankowskii*, and two pairs were mixed. The ranges of *P. p. leucoptera* and *P. s. jankowskii* overlap considerably during seasonal migrations and wintering in both the Kherlen and Argun River basins. However, the minimum distance from the nest of a pure pair of *P. p. leucoptera* to the nest of a pure pair of *P. s. jankowskii* at the time of our study was 70 km, indicating that the breeding areas of these species are not yet in full contact. However, the minimal distance from the nest of a mixed pair to the nest of a homospecific pair of *P. p. leucoptera* was 4 km. According to our long-term field observations, as well as reports of local residents, contact between the ranges of *P. s. jankowskii* and *P. p. leucoptera* in Eastern Transbaikalia began only 20–30 years ago. Contact in Mongolia most likely began much earlier, according to literature and other data sources (Goroshko et al., 2018). These differing timelines likely relate to differences in the scale of hybridization at these two contact sites.

In our study of viability, some fertile pairs in all populations had non-viable eggs in nests in addition to viable eggs or chicks. These included 22% of nests in Central Transbaikalia (5 nests out of 23); 11% of nests in Eastern Transbaikalia (3 out of 28), and 47% of nests in Mongolia (9 out of 19) (Table 1). The contents of all non-viable eggs were examined. In most cases, they were without signs of embryo development. Less often they contained embryos that died at the early stages of development, and extremely rarely contained embryos that died at the middle stages of development. Most notably, the complete clutches of some pairs contained only non-viable eggs, without any sign of embryo development, indicating a sterile pair. In Mongolia, 14 (42%) of 33 nests belonged to sterile pairs, while the remainder of pairs were fertile because their clutches contained chicks or at least some viable eggs at the final stage of incubation. Only one nest with a non-viable clutch was found in Eastern Transbaikalia (3% of 29 nests). No sterile pairs were identified in Central Transbaikalia. Differences are statistically significant when comparing the hybrid population of Mongolia with the "pure" control population of Central Transbaikalia (Table 1).

It is important to note that in Mongolia, 12 of the 14 nests with completely unviable clutches were found in the eastern part of the Kherlen River valley, where most mixed pairs were found. Among these 14 unfertile pairs, at least 6 involved a hybrid bird. In Eastern Transbaikalia, of the two mixed pairs mentioned above, the first consisted of a hybrid female (based on coloration and intermediate alarm signal) and a male *P. p. leucoptera*. For this pair, all 8 eggs were non-viable. The second mixed pair consisted of a female *P. s. jankowskii* (according to the mtDNA of her chicks) and a male *P. p. leucoptera* (according to his phenotype and voice). This pair produced five live chicks. The 19 pure pairs of *P. p. leucoptera* and *P. s. jankowskii* in the Eastern Transbaikalia were fertile. Twenty-three pure pairs of *P. p. leucoptera* in Central Transbaikalia and four pairs of *P. s. jankowskii* in China were also fertile.

DISCUSSION

The data we have obtained clearly indicate a lower rate of breeding success, at least at the stage of nesting, in the hybrid magpie population in eastern Mongolia compared to that of the relatively pure nearby populations of Central and Eastern Transbaikalia, and China, which experience similar environmental conditions.

Table 1. Nesting pair composition and clutch viability in different populations of magpies

Population	Number and composition of pairs producing nests surveyed					Fertility of pairs			
	number of nesting pairs	number of nesting pairs of pure *P. p. leucoptera*	number of mixed pairs or pairs involved hybrid	number of nesting pairs of pure *P. s. jankowskii*	species of one of pair members not detirmined	number of viable clutches of the fertile pairs	number of non-viable clutches with all died eggs	number of viable clutches with part of died eggs	total number of clutches with part or all died eggs
P. p. leucoptera, Central Transbaikalia	23	17	0	0	6	23	0	5	5
P. p. leucoptera + *P. s. jankowskii*, Eastern Transbaikalia	29	9	2	10	8	28	1	3	4
P. p. leucoptera × *P. s. jankowskii*, Northeastern Mongolia	33	7	11	0	15	19	14*	9*	23*
P. s. jankowskii, Northeastern China	4	0	0	4	0	4	0	0	0
Total	89	33	13	14	29	74	15	17	32

* Signifies a significant difference in χ^2 values ($p < 0.05$) between the hybrid population in Mongolia and the control population in Central Transbaikalia.

Nesting failure in the Mongolian population is attributed to problems with fertilization and/or embryonic mortality. As logistical constraints prevented us from performing checks at all stages of nesting, and because the determination of one or the other of these causes was not among our research goals, this topic should be the subject of future investigation. While non-viability of eggs can result from a number of causes, including non-fertilization and embryo death due to genetic problems, but also unfavorable environmental conditions (e.g., hypothermia, extreme weather, intense disturbance). However, we observed no external causes that would have killed embryos in the nests that failed.

While genetic analysis (mtDNA and nuclear SNPs) was conducted using samples taken from live chicks from these nests in our previous work, the publication of which also specifies the nest locations (Kryukov et al., 2022), these analyses were not possible for eggs which did not contain embryos. In our nuclear SNP analysis of live chicks from nests including a hybrid parent (Kryukov et al., 2022), we found unequal proportions of species-specific alleles in several remote broods. This means that at least some of the first generation (F1) hybrids are viable and fertile, which provides presence of a number of generations of backcrosses.

Hybridization is often accompanied by sterility and/or decreased viability of the first or subsequent generations of hybrids. Hybridological analysis is applied in the consideration of species status of animals such as rodents (Sokolov and Vasilyeva, 1993; Osipova and Soktin, 2006), although it is often insufficient as a sole criterion. Besides, experimental crossing in captivity can present a misleading understanding of natural processes, as behavioral prezygotic reproductive barriers (e.g. differences in mating behavior and mate choice) and the species' ecological preferences are not accounted for. In natural hybrid zones, lower fertility or fitness of hybrids indicates existence of a "tension zone" (Barton and Hewitt, 1985), which is maintained by equilibrium between selection against hybrids and gene flow towards the hybrid zone. From the other hand, narrowness of a hybrid zone such as that observed between orioles *Icterus bullockii* and *I. galbula* (Walsh et al., 2020) suggests the presence of selection against hybrids. Analogously, lower clutch sizes have been recorded at the narrow hybrid zone between grosbeaks *Pheucticus ludovicianus* and *P. melanocephalus* (Anderson and Daugherty, 1974).

Particularly interesting are cases of partial hybrid sterility under conditions of incomplete speciation. Postzygotic interspecies isolation is less strongly exhibited in birds than in other vertebrates, such that crosses within an avian genus are often successful not only in captivity, but in the wild, and this insignificantly reduces fertility in F1. However, hybridization among wild birds occurs more seldom, than might be expected because prezygotic isolation plays a significant role in maintaining species integrity (Grant, P. and Grant, B., 1992; Edwards et al., 2005). Since postzygotic isolation takes longer to develop than speciation usually requires, prezygotic isolation is particularly important in early stages of the speciation process (Price and Bouvier, 2002). Nevertheless, this isolation is quite often broken, particularly in conditions where the choice of conspecific mating partners is limited, such as captivity, at the borders of semi-sympatric ranges, and at invasion fronts.

From 10 to 20% of bird species hybridize in nature, though the estimated percentage varies depending on the oft-disputed taxonomic ranks of the crossing forms, whether species or subspecies (Ottenburghs, 2023). Results of 407 different hybrid crosses have been reported for 367 bird species, ranked from subspecies to families. These pairings have produced offspring with varying reproductive success, from normal fertility to complete inviability of both sexes (Price and Bouvier, 2002). More than half of these crosses within genera produced fertile hybrids, providing evidence that in the course of speciation, prezygotic isolation develops earlier than postzygotic isolation, and sterility of F1 individuals appears before inviability. Infertility of F1 hybrids was observed in both sexes, but in 98% of cases males were more viable than females. The infertility of hybrids has been found to positively correlate with genetic divergence within the mitochondrial *cytB* gene (Price and Bouvier, 2002). It is interesting that often, closely related species exist in sympatry, even when genetically capable of producing viable F1.

Analysis of the results of interspecies crossing in captivity of 21 species of Columbidae pigeons has found that with increasing divergence (as measured by genetic distance of the mitochondrial *cytB* gene), the proportion of non-viable eggs per clutch increases (Lijtmaer et al., 2003). The rate of postzygotic isolation is similar for the first generation of hybrids and backcrosses, but is elevated in the second hybrid generation. In F1 hybrids resulting from interspecies crossings in captivity of South American finches in the genus *Sporophila,* 71% of chicks hatched, while in conspecific pairs only 44% (the latter probably due to inbreeding in captivity), but the number of fledglings did not differ between these two groups (Campagna et al., 2018).

Though much research on natural hybridization of birds does not investigate the fertility of hybrids, or did not find it to decrease, there are some examples of similar findings. In hybridizing subspecies of red-billed toko *Tockus erythrorhynchus,* hatching success in hybrid or heterospecific pairs is reduced by 10% compared to homospecific pairs with the same clutch size (Delport et al., 2004). Breeding success in hybridizing chickadees *Parus atricapillus* and *P. carolinensis* correlates positively with the index of genetic compatibility of the nesting partners, and pure males are more productive than hybrids (Bronson et al., 2005). In the hybrid zone of flycatchers *Ficedula hypoleuca* and *F. albicollis,* hybrid females are partially fertile, and clutches of pairs with such females had more non-viable eggs than the clutches with hybrid males (Sætre et al., 1999). Moreover, hatching success in the pairs with hybrid males was either complete, or zero. Breeding success of hybrids between buntings *Passerina amoena* and *P. cyanea* in the hybrid zone is reduced; the proportions of hatched chicks and fledged fledglings is lower in the pairs involving hybrid females than in those involving hybrid males (Baker and Boylan, 1999). These and many other examples confirm Haldane's rule (Haldane, 1922), which posits reduced fitness and fertility in hybrids of the heterogametic sex (for birds, the female). Due to constraints of our methodology, including difficulties in visual identification of the sexes, we could not verify possible differences in sex ratio between populations. However, the asymmetric nuclear gene introgression from *P. s. jankowskii* to *P. p. leucoptera* without corresponding mtDNA introgression which we recorded in this population (Kryukov et al., 2022) might be explained by the partial female F1 sterility expected according to Haldane's rule.

The postzygotic isolation described in these examples reflects a degree of genome incompatibility manifested by compromised fertility and/or viability of F1 hybrids, their reduced ecological fitness, and genetic incompatibility in F2 and backcrosses. Consequences of postzygotic isolation also include the possibility of lowered fitness due to genetic constraints, challenges in mating choice and breeding, and genetic effects in subsequent hybrid generations. For example, in the center of a wide sympatric hybrid zone of buntings *Emberiza citrinella* and *E. leucocephalos*, where the proportion of hybrids is highest, the breeding success of all pairs is similar, but hybrids have reduced longevity (Rubtsov, 2021). The production of non-viable eggs may indicate a lack of copulation, or fertilization, or failure in embryonic development. In the case of our study, the possibility of infertilized eggs is excluded, because all nests under observation had two parents. Thus the breeding failure noted in our study could be caused by impaired fertilization, death at early stages of embryogenesis, or various anomalies in the hybrid offspring. The source of endogenous barriers to hybrid incompatibility is often explained by models such as that of Bateson–Dobzhansky–Muller, based on epistatic interaction of loci (Orr, 1996). Chromosomal rearrangements, genome restructuring, divergence of protein-coding genes and noncoding DNA, appearance of indels, transposon activation, and other processes may also be among the causes (Maheshwari and Barbash, 2011; Abbott et al., 2013).

Cytogenetic mechanisms of hybrid sterility are fairly well understood. Meiotic disorders result in partial or complete sterility due to the death of gametes, or unbalanced gametes, the causes of which may be a variety of chromosomal rearrangements and gene differences. Thus, in hybrids of voles *Microtus arvalis* and *M. levis*, sterility occurs as a result of disruption of synapsis and the repair mechanism of DNA double strand breaks (Torgasheva and Borodin, 2016). In an example of birds, hybrids between musk duck *Cairina moschata* and white Peking duck are sterile due to differences in the 1st and 4th pairs of chromosomes, which prevents their synapsis and stops the 1st phase of meiosis (Mott et al., 2004).

The molecular basis of incompatibility is poorly explored. The search for "speciation genes" is ongoing. Candidate genes are proposed to be either individual loci distributed throughout the genome or islands of increased divergence (speciation islands), which do not introgress across hybrid zones (Turner et al., 2005; Ellegren et al., 2012; Ottenburghs et al., 2017). In the European hybrid zone of the house mouse subspecies *Mus musculus musculus* and *M. m. domesticus*, F1 hybrid males are sterile, for which five autosomal loci are responsible that are polymorphic between populations (Larson et al., 2018). In the hybridization of two species of nightingales, *Luscinia megarhynchos* and *L. luscinia,* females are sterile, which is associated with genomic islands of high divergence (Mořkovský et al., 2018). These islands contain genes responsible for female meiosis and metabolic genes, and are characterized by a high level of linkage disequilibrium and low level of recombination. In successful backcrosses, introgression, including adaptive introgression, usually occurs (Currat et al., 2008; Hedrick, 2013). Limited asymmetric introgression of nuclear SNPs and its absence in mitochondrial genes was registered in our previous study of this magpie contact zone (Kryukov et al., 2022). In the case of selection against hybridization, selectivity in mate choice may develop and be reinforced (Dobzhansky, 1940; Servedio and Noor, 2003) not only by prezygotic isolation (Sætre et al., 1997), but also postzygotic isolation (Irwin, 2020), thus increasing divergence of the parent species. Within our study system, under conditions of the reduced breeding success of heterospecific pairs and hybrids, the formation of positive assortative mating and species divergence in behavior may be expected. These species under study differ in their vocalizations (Kryukov et al., 2017, 2022), and this difference may be enhanced by selection against hybridization. However, it has been shown that only sufficiently intense selection is required for the development of prezygotic isolation (Liou and Price, 1994), which is not obvious in our case.

Considering the propensity of both species to expand their ranges, there may be a gradual expansion of the zone of contact and hybridization, but without increasing bird densities in hybridogenic populations, given the evidence of selection against hybridization and limited environmental resources. Over time, we may expect the formation of a "tension zone" formed of several patches and thus corresponding to the concept of a "mosaic hybrid zone" (Harrison, 1986). Biotopes in this magpie contact zone consist of patches of suitable breeding habitat composed of river and lake valleys partly overgrown with bushes and low-growing trees, separated by vast, unsuitable steppe areas. Due to differences in the timeline of contact initiation, the numerical ratio of founder forms, and varying ecological conditions in different parts of the hybrid zone, the pattern of hybridization may not be uniform across this area, and its growth may result in a variety of indirect consequences. These are difficult to predict, because secondary contact between closely related species is influenced by such a wide array of exogenous and endogenous factors.

CONCLUSIONS

Recent contact between two closely related, but genetically diverged magpie species, for which we first describe incomplete reproductive isolation, provides unique opportunities to monitor the initial stages of the introgression process, possible selection against hybridization, and increased divergence, which together will determine the further fate of this secondary contact and the integrity of both species.

The foreword to the special issue of the journal *Molecular Ecology* on the genomics of hybridization concludes that: "Very few studies ... combine genomic analyses, with measures of fitness of admixed individuals under common garden conditions or in the wild. In studies of hybrid zones, in particular, this should be attempted whenever possible, ... as a complement to genomic analyses, so as to obtain a more complete understanding of the factors that may allow species to originate and be maintained in the face of gene flow" (Abbott et al., 2016, p. 2330). Our work partially fills this gap.

ACKNOWLEDGMENTS

We are much grateful to Dr. M. Kessler for editing the English.

FUNDING

The research was carried out within the governmental work program of Ministry of Science and Higher Education of the Russian Federation (theme no. 121031500274-4) and State work program of Institute of Nature Resources, Ecology and Cryology, Siberian Branch of the Russian Academy of Sciences.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Studies were conducted without removing viable eggs or chicks.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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