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Experimental evidence for ovarian hypofunction in sparrow hybrids

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Abstract

Background: Postzygotic isolation in the form of reduced viability and/or fertility of hybrids may help maintain species boundaries in the face of interspecific gene flow. Past hybridization events between house sparrows (*Passer domesticus*) and Spanish sparrows (*P. hispaniolensis*) have given rise to a homoploid hybrid species, the Italian sparrow (*P. italiae*). Although genetic incompatibilities are known to isolate these three species, the biological consequences of these incompatibilities are still unknown in early generation hybrids.

Methods: We investigated whether F1 hybrids between house and Spanish sparrows experience reduced viability or fertility. More specifically, we generated hybrids through controlled crosses in aviaries, and compared ovaries of female hybrids with female of pure-species sparrows.

Results: We found that overall, hybrid ovaries were underdeveloped and that half of all female hybrids exhibited symptoms of ovarian hypofunction (ovarian atrophy and complete absence of developed follicles).

Conclusions: Fertility in hybrids is a common consequence of post-zygotic barriers between species. We discuss these results in light of previous findings on genetic incompatibilities between the parent species and the potential role of incompatibilities in hybrid speciation, a rare evolutionary process in birds.

Keywords: Hybrid fitness, Hybridization, Ovary, Sparrows, Speciation

Background

As divergence in allopatry between two species increases, levels of postzygotic isolation are also likely to increase (Coyne and Orr 1997). Extrinsic sources of postzygotic isolation, when hybrids are ecologically or biologically unfit, are thought to play an important role in the early stages of population divergence, while intrinsic genetic incompatibilities causing hybrid sterility and/or inviability are thought to develop later (Presgraves 2010; Seehausen et al. 2014). However, little is known about postzygotic isolation between pairs of species for which past hybridization has generated a new species that is reproductively isolated from both parents (a process called hybrid speciation; Mallet 2007). Such information is critical if we want to understand which isolating

mechanisms may constrain or facilitate hybrid speciation (Abbott et al. 2013; Schumer et al. 2014). It has been hypothesized that postzygotic barriers may hinder the hybrid speciation process as they will, by necessity, involve the purging of genetic incompatibilities in early generation hybrids (Nolte and Tautz 2010). However, if instead these incompatibilities can be sorted in such a way that one subset of the genes function as reproductive barriers against one of the parents and a different subset against the other parent (Trier et al. 2014), they may favor the emergence of a fully isolated new hybrid lineage (Hermansen et al. 2014; Trier et al. 2014).

The house sparrow (*Passer domesticus*) and Spanish sparrow (*P. hispaniolensis*) are two closely related, ecologically similar passerine species. However, differences in habitat and timing of breeding appear to represent pre-mating barriers to gene exchange (Summers-Smith 1988; Hanh Tu 2013). In contrast, there is currently no evidence for post-mating prezygotic isolation (Cramer

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et al. 2014). Past episodes of hybridization between house sparrows and Spanish sparrows have resulted in the formation of a homoploid hybrid species, the Italian sparrow (*P. italiae*) (Elgvin et al. 2011; Hermansen et al. 2011). Further, the two parent species are still sympatric in many parts of their range and occasionally hybridize (Summers-Smith 1988; Hermansen et al. 2014). There is also molecular evidence that these two species are isolated by Z-linked genetic incompatibilities (Hermansen et al. 2014). However, little is known about the biological consequences of these genetic incompatibilities and whether they are of an extrinsic and/or intrinsic nature.

Haldane's rule predicts that the heterogametic sex should suffer more from genetic incompatibilities (Haldane 1922). In birds, the female is the heterogametic sex (ZW) and is hence predicted to suffer more from intrinsic incompatibilities than male hybrids. Here, we investigate whether hybrid females between house sparrows and Spanish sparrows exhibit reduced fertility (through ovarian hypofunction indicated by ovarian atrophy and/or complete lack of follicular development) by dissecting and measuring the ovaries from experimental interspecific crosses between these species.

Methods

We used captive populations of the two species to form experimental crosses in a common garden environment. We captured house sparrows in Oslo, Norway (59.934°N, 10.723°E), and Spanish sparrows in Badajoz, Spain (38.649°N, 7.215°W); most birds were captured in 2010, and breeding activity was monitored the following years. Sparrows were maintained in mixed-sex aviaries housing 15–18 pairs of birds. Although there is no known information about the pedigrees of these different aviaries for logistic reasons, inter and intraspecific crosses were controlled through the following design: two aviaries contained males and females from a single species, while two other aviaries contained males from one species and females from the other species. Hence, hybrids sired from both types of parental combinations, as well as pure house and Spanish sparrows, were generated in a controlled fashion.

The birds were fed *ad libitum* with seeds and dried insects, and were supplemented with mealworms during the breeding season. We provided nest boxes and nesting material and allowed the birds to breed and pair without interference. A number of birds in all four aviaries bred successfully, and eggs were laid between April and September each year. During the breeding season (May) of 2014, on the same day, we sacrificed 6 hybrid females (5 fathered by a house sparrow male and Spanish sparrow female and 1 fathered by a Spanish sparrow male and house sparrow female) and 12 pure species females (8 house and

4 Spanish sparrows) and inspected their reproductive organs. All birds were mature adults (i.e. at least 2 or more years of age, either wild caught or aviary-born) and had been maintained in captivity under identical conditions for at least two years prior to the current experiment.

Prior to dissection, we measured tarsus length and beak height with a caliper to the nearest 0.1 mm, wing length with a ruler to the nearest 0.5 mm, and body mass with a Pesola balance to the nearest 0.1 g. Ovaries from a total of 18 adult females were scored as either normally developed or atrophied (the main symptom of ovarian hypofunction) and stored in RNA-later buffer for future transcriptomic work. We measured length and width for each ovary with a digital caliper to the nearest 0.1 mm and calculated their volume based on the assumption that ovaries are ellipsoids with equal height and width. We also inspected each ovary for the presence of pre-ovulatory follicles and counted the number of pre-ovulatory follicles if any were present. We took photographs of one normally developed ovary from a pure house sparrow female and one atrophied ovary from an F1-hybrid female (cross between a house sparrow sire and a Spanish sparrow dam). We performed an ANOVA to test for significant differences in ovary volume between hybrid and pure-species females and also between species. We also investigated whether the ovary width or length of some hybrid females (i.e. those that exhibited atrophied ovaries) were significant statistical outliers compared to the distribution of pure-species ovary widths or lengths using the Z-score method. A Fisher's exact test was performed to determine whether the proportion of individuals with ovarian hypofunction was elevated in hybrids relative to pure individuals. Furthermore, to investigate whether potential differences in follicle development between normal and potentially atrophied ovaries were not only physical but also functional, we used an ANOVA to test for species and hybrid differences in the number of pre-ovulatory follicles. We then conducted a one sample *t* test to determine whether the average number of pre-ovulatory follicles found in normal ovaries, irrespective of species, was significantly different from 0 (the number found in all atrophied ovaries).

Results

All individuals in the study had morphological features within their respective species ranges: F1 hybrids did not differ from either parent species in tarsus or wing length, beak height or body mass (Table 1). However, on average, hybrid females had significantly smaller ovaries than pure species females ($F_{1,17} = 5.64$; $p = 0.030$; pure species volume: mean: 1202.7 mm³, SD: 472.8; hybrid volume: mean: 531.0 mm³, SD: 729.8), while there was no significant difference in ovarian volume between house and Spanish

Table 1 Morphometric measurements of the two parent species and F1 hybrids

Species	<i>P. domesticus</i>	Hybrid	<i>P. hispaniolensis</i>
Tarsus length (mm)	19.0 (1.0)	19.4 (0.4)	19.7 (0.5)
Wing length (mm)	77.6 (2.6)	76.3 (0.9)	78.3 (0.3)
Weight (g)	27.4 (2.8)	26.9 (3.8)	27.9 (3.5)
Beak height (mm)	7.87 (0.3)	7.93 (0.2)	8.03 (0.4)

Mean and standard deviation (in parentheses)

sparrow females ($F_{1,11} = 1.85$; $p = 0.20$) (Fig. 1a, b). Furthermore, three hybrid females (all fathered by a house sparrow male and Spanish sparrow female) were significant outliers (Fig. 1a; $p = 0.05$) in terms of ovary width and length, with surprisingly narrow and short ovaries, suggesting severe atrophy, which was not the case for any pure species females. This meant that 50 % of the hybrid females ($n = 6$) had atrophied ovaries, which were characterized by a drastic reduction in size (Fig. 1a, d) compared to pure house sparrow ($n = 8$) and Spanish sparrow females ($n = 4$).

This difference represents a significantly higher incidence of ovarian atrophy in hybrids ($p = 0.025$; Fig. 1d). Moreover, prehierarchical follicles were detected in all ovaries scored as normal, whereas none could be detected in ovaries scored as atrophied (Table 2), which suggests that atrophied ovaries differ both physically and functionally from normal ovaries. Additionally, in a high proportion of individuals with a normal ovary (pure species: 7/12; hybrids: 3/3), pre-ovulatory follicles could be observed (although in the three hybrid individuals with normal ovaries only one pre-ovulatory follicle could be observed), unlike in individuals with an atrophied ovary (0/3) (Table 2). The number of pre-ovulatory follicles in pure species females was also not significantly different from hybrids with normal ovaries (ANOVA: $F_{1,14} = 7.86$; $p = 0.112$) but the average number of pre-ovulatory follicles found in normal ovary individuals (mean: 1.47, standard deviation: 1.35) was significantly different from 0 (one-sample t test: $t = 4.19$; $p = 0.001$) and hence from what was found in hybrid individuals with atrophied ovaries.

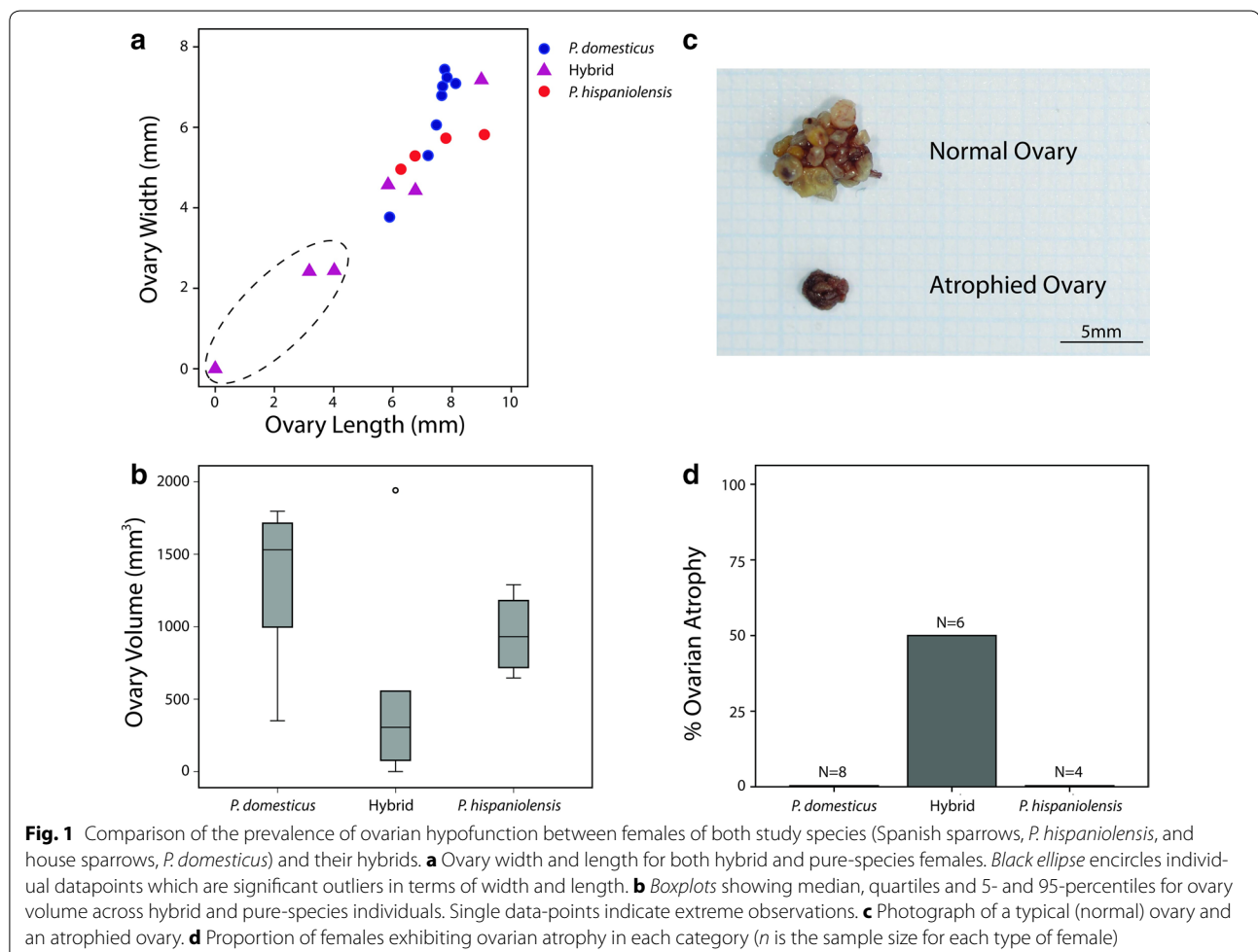


Table 2 Presence of immature and pre-ovulatory follicles in atrophied ovaries versus normal ovaries

Ovary type	Normal (n = 15)	Atrophied (n = 3)
Presence of prehierarchal follicles (%)	100	0
Presence of pre-ovulatory follicles (%)	66.7	0
Average number of pre-ovulatory follicles	1.47	0

Discussion

Our finding of underdeveloped or atrophied ovaries, combined with the complete absence of follicular development in otherwise phenotypically normal and viable adult hybrid females, is symptomatic of ovarian hypofunction, and suggests that these individuals were unable to produce ova (Oguntunji and Alabi 2010). Anecdotal, and consistent with Haldane's rule (Haldane 1922), two hybrid males produced during the experiment exhibited normal sperm function (i.e. motility) relative to individuals from both pure species (Cramer et al. 2015). Strong postzygotic barriers between the house sparrow and Spanish sparrow may seem surprising given that historical hybridization between the two species led to the formation of the hybrid Italian sparrow (Hermansen et al. 2011). Although as many as 9 % of bird species are known to hybridize (Grant and Grant 1992; Mc Carthy 2006), the Italian sparrow is the only bird species that has been shown to be of hybrid origin and to have developed reproductive barriers against both parent species (Hermansen et al. 2011; Trier et al. 2014; but see Brelford et al. (2011) for another case of an avian taxon of hybrid origin). Thus, there appear to be strong constraints on hybrid speciation in birds. Postzygotic isolation mechanisms may represent such an obstacle (Nolte and Tautz 2010), but these appear to evolve rather slowly in birds (Grant et al. 1996; Price and Bouvier 2002). Recently, Trier et al. (2014) found that genetic incompatibilities isolate the Italian sparrow from its parent species. Furthermore, these genetic incompatibilities represent a subset of those found to isolate house and Spanish sparrows where they occur in sympatry (Hermansen et al. 2014). Identifying the physiological effects of these incompatibilities and determining if these are extrinsic (leading to maladaptation in hybrids) or intrinsic (leading to inviable or infertile hybrids) increases our understanding of mechanisms acting during hybrid speciation.

Here, we experimentally confirm that intrinsic genetic incompatibilities isolate the house and Spanish sparrows, and that these manifest as an increase in the incidence of ovarian hypofunction in hybrid individuals. Interestingly, previous genetic studies have shown that

genetic incompatibilities between these two species are predominantly mito-nuclear and sex-linked (Trier et al. 2014). Sex-linked incompatibilities have also been found to lead to hybrid sterility in many other systems (Payseur et al. 2004; Qvarnström and Bailey 2008). Trier et al. (2014) identified six Z-linked candidate incompatibility loci between the hybrid Italian sparrow and either of the parent species. Evidence suggests that these loci have been sorted during the hybrid speciation process in such a way that one subset of the genes function as reproductive barriers against the Spanish sparrow, and a different subset against the house sparrow (Hermansen et al. 2014; Trier et al. 2014). One of these six loci associated with reproductive isolation between the Italian and Spanish sparrow (Trier et al. 2014) as well as between the house and Spanish sparrow (Hermansen et al. 2014) is situated in the coding region of the gene *GTF2H2*, a transcription factor highly expressed in oocytes (Kakourou et al. 2013). This gene has been shown to be involved in premature ovarian hypofunction (Aboura et al. 2009) and polycystic ovary syndromes (Haouzi et al. 2012) in other vertebrates. If *GTF2H2* is indeed contributing to ovarian hypofunction in sparrow hybrids, it may have been instrumental in this hybrid speciation event. In the future, mapping the occurrence of ovarian atrophy to candidate genes such as *GTF2H2* in sparrow hybrids would yield the ultimate link needed to establish the importance of parental incompatibility sorting during hybrid speciation. Hence, more experimental work should be conducted on the biological consequences of postzygotic barriers to unravel the genetic predispositions to hybrid speciation.

Availability of supporting data

The data set supporting the results of this article will be available in the DRYAD repository [unique persistent identifier and hyperlink to dataset(s) in <http://format/>], upon acceptance.

Authors' contributions

FE, FH, JSH, GPS carried out the breeding experiment. FE, MR, AR and ERAC carried out or assisted with the dissections, AR measured the ovaries, MR evaluated follicular activity. FE and ERAC conducted the statistical analyses. FE, MR, AJ and GPS participated in the design of the study and its coordination. FE wrote the manuscript and all authors helped to draft it. All authors read and approved the final manuscript.

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This study is the result of a collaboration between two research groups: the Sparrow Group at the Department of Biosciences (University of Oslo) and the Sex and Evolution Research Group at the Natural History Museum (University

of Oslo) and was initiated by GPS as part of large research project on hybridization in *Passer* species.

Acknowledgements

We thank the numerous assistants who helped in the field and during the experimental phase of this project. This study was funded by the Research Council of Norway (GPS, AJ), the Swedish Research Council (FH, FE, AR), the University of Oslo (JSH) and the Marie-Curie Foundation (FE). All authors declare that the present study complies with the current laws and ethical standards of animal research in Norway. Ethical permission was issued to F. Haas and M. Rowe (Norwegian Animal Research Authority—FOTS ID 2394 and Norwegian Animal Research Authority—FOTS ID 6323).

Competing interests

The authors declare that they have no competing interests.

Received: 16 September 2015 Accepted: 12 February 2016

Published online: 24 February 2016

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