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Multiple Sexual Signals and Behavioral Reproductive Isolation in a Diverging Population

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ABSTRACT: Sexual trait divergence has been shown to play a role in the evolution of reproductive isolation. While variation in multiple sexual signals is common among closely related species, little is known about the role of these different axes of phenotype variation with respect to the evolution of behavioral reproductive isolation. Here we study a unique population of barn swallows (*Hirundo rustica transitiva*) that can be distinguished phenotypically from its neighboring populations only on the basis of two features of male plumage: exaggerated expression of both long tail streamers and dark ventral coloration. Using phenotype manipulation experiments, we conducted a paternity study to examine whether both traits are sexually selected. Our results show that an exaggerated form of the local male phenotype (with both tail elongation and color darkening) is favored by local females, whereas males whose phenotypes were manipulated to look like males of neighboring subspecies suffered paternity losses from their social mates. These results confirm the multiple signaling role of the unique tail and color combination in our diverging population and suggest a novel possibility according to which multiple sexual signals may also be used to discriminate among males from nearby populations when prezygotic reproductive isolation is adaptive.

Keywords: multiple sexual signals, speciation, prezygotic isolation, barn swallow.

Introduction

Sexually selected traits have been suggested to play a major role in promoting prezygotic reproductive isolation during population divergence and speciation (Seehausen et al. 1997; Masta and Maddison 2002; Boul et al. 2007). Most populations use multiple sexual signals in the process of reproductive and competitive encounters (Candolin 2003; Bro-Jørgensen 2010), and it has been previously suggested that multiple trait divergence may play a role in the evo-

lution of population divergence in geographic isolation (Bailey et al. 2007). However, despite the ubiquity of multiple sexual signals, the mechanisms underlying their evolution within populations and their link to speciation are still poorly understood (Bro-Jørgensen 2010; Uy and Safran 2013).

Among several possible mechanisms, two adaptive processes have been featured prominently as explanations for patterns of sexual trait divergence in closely related populations. First, reinforcement or reproductive character displacement may be adaptive if strong selection against hybrids exists (Dobzhansky 1940; Butlin 1987; Ritchie 2007; Ortiz-Barrientos et al. 2009). Another possibility is that sexual selection shapes trait variation in each population differently as a result of stochastic events or different environmental contexts, thereby promoting prezygotic reproductive isolation between diverging populations (Schluter and Price 1993; Boughman 2001; Pauers et al. 2004; Seddon 2005; Boul et al. 2007; Ritchie 2007; Seehausen et al. 2008; Wilkins et al. 2013). However, these two mechanisms are not mutually exclusive (Ritchie 2007).

While most studies of prezygotic reproductive isolation through mate selection have addressed the use of a single trait or signal as a mating cue (e.g., Saetre et al. 1997; Hoskin et al. 2005), there is growing recognition that divergence in multiple sexual signals may play an important role in speciation (Seddon et al. 2008; Bro-Jørgensen 2010; Uy and Safran 2013). Multiple sexual signals are usually thought to evolve to back up the information content of a single signal or to convey multiple messages by advertising different aspects of male quality (Candolin 2003; Doucet and Montgomerie 2003; Chaine and Lyon 2008; Bro-Jørgensen 2010; Hohenlohe and Arnold 2010). The link between divergence in multiple sexual signals and speciation may also be explained by trait and preference divergence in different ecological contexts (Schluter and Price 1993; Candolin 2003). An additional but less well studied possibility is that female preferences for multiple

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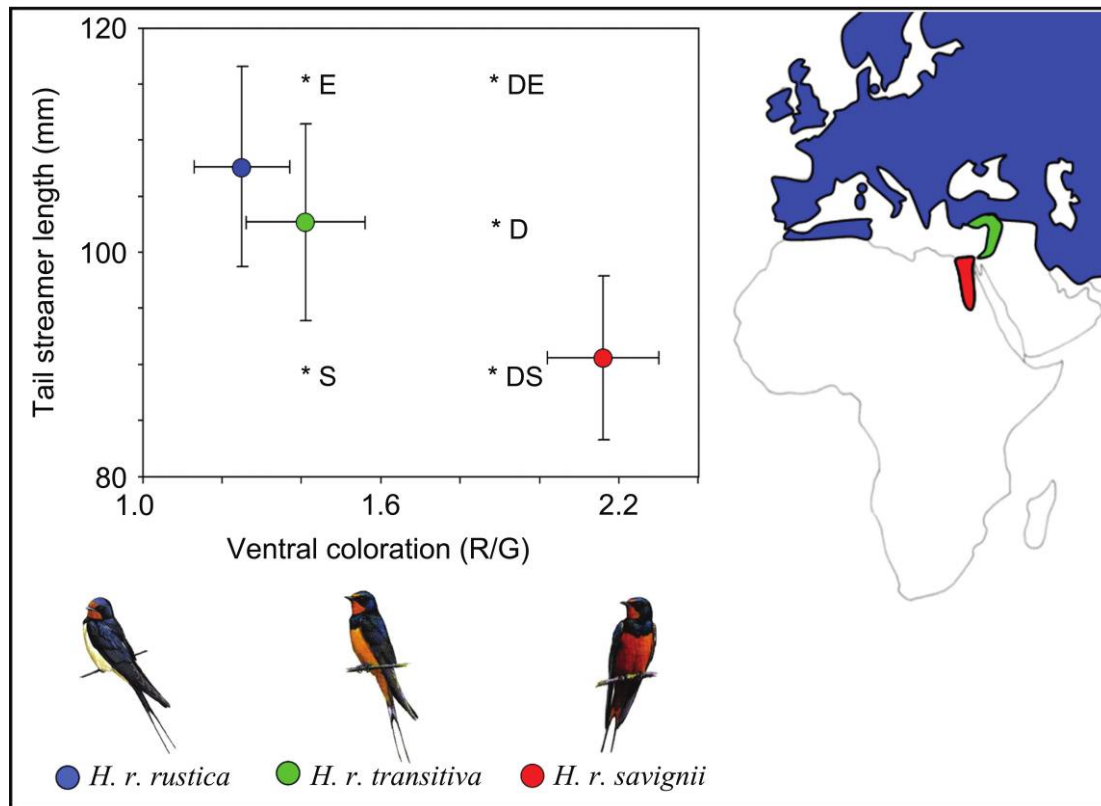


Figure 1: Trait distribution (see table 1 for details) and breeding distribution of the European (*Hirundo rustica rustica*), east Mediterranean (*H. r. transitiva*), and Egyptian (*H. r. savignii*) populations of barn swallow (the distribution of *H. r. rustica* expands beyond the present map). Error bars mark the standard deviation. Asterisks demonstrate the amount of sexual ornament modification of the various treatment groups (E and S: 13-mm elongation or shortening of tail streamers, respectively; D: enhancement of 0.45 R/G ratio in ventral coloration) with respect to *H. r. transitiva* phenotypic mean. *Hirundo rustica transitiva* is currently parapatric with respect to the *H. r. rustica* subspecies and allopatric with respect to the *H. r. savignii* due to their current separation by the Sinai Desert (however, this geographic barrier has repeatedly retreated and been reintroduced in three separate events during the end of the Pleistocene; Goodfriend and Magaritz 1988). Art by Hilary Burn and used with her permission.

sexual signals may be a solution to the problem of conspecific recognition, particularly in cases in which closely related populations have overlapping distributions (Pfenig 1998; Hohenlohe and Arnold 2010) or when a population is itself a hybrid of two adjacent ones (Mallet 2007). In such cases, conditioning mate choice on the expression of a unique combination of multiple signals (rather than on one signal alone) may further facilitate or maintain prezygotic reproductive isolation, but to the best of our knowledge such a process has never been documented.

To examine the evolutionary forces driving multiple sexual signals and to explore their possible effect on population divergence and speciation, we studied a population of the east Mediterranean barn swallow (*Hirundo rustica transitiva*), which is one of six known subspecies of the

barn swallow subspecies complex (Turner and Rose 1989; Møller 1994). This population of sedentary barn swallows breeds along the east coast of the Mediterranean and resides south of the distribution of the migratory European population (*H. r. rustica*) and north of the sedentary Egyptian population (*H. r. savignii*; see fig. 1). These populations are closely related (Dor et al. 2010, 2012) yet phenotypically divergent from one another in at least two prominent features of morphology (see fig. 1; data available in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.g8n63>; Vortman et al. 2013).

The European population (*H. r. rustica*) is well studied, and its males are characterized by pale ventral coloration and long, sexually dimorphic tail streamers that have been shown to function as a costly sexual signal (Møller 1988;

Møller and de Lope 1994; Saino et al. 1997). Experimental manipulations of tail streamer length in this population (without alteration of other traits) reduced the length of the pre mating period and favorably increased the number of extrapair copulations and the degree of male within-pair and extrapair paternity (Møller 1988; Saino et al. 1997). Males of the sedentary Egyptian population (*H. r. savignii*) are characterized, on the other hand, by dark chestnut ventral coloration and relatively short tail streamers (Møller 1994; fig. 1; table 1; appendix, available online). While the sexual signals of *H. r. savignii* have not been studied, this subspecies shows the highest level of sexual dimorphism with respect to ventral coloration and the lowest level of sexual dimorphism with respect to tail streamer length (Vortman 2013; see appendix). Males of the east Mediterranean population (*H. r. transitiva*) exhibit an intermediate combination of these two signals, having both long tail streamers and dark ventral coloration. In *H. r. transitiva* the expression of each of these traits is correlated with social and genetic measures of male reproductive success (Vortman et al. 2011), suggesting that both traits may serve as sexual signals in this population.

The goals of this study were to test experimentally whether elongated tail streamers and dark ventral coloration are indeed sexually selected in *H. r. transitiva* and to clarify their potential combined effect as multiple signals in light of the unique position of *H. r. transitiva* in the *rustica-transitiva-savignii* clade (fig. 1). To test causal relationships between male signal traits and female preference, we manipulated tail streamer length and ventral color, two aspects of the phenotype known to be involved in mate choice in two different barn swallow populations (Møller 1988; Safran et al. 2005). The phenotype manipulations were carried out between successive broods, and their impact was measured by analyzing the change in a male's paternity in his first brood (premanipulation) and second brood (postmanipulation), thus enabling us to identify whether a female allocated paternity differently to her social mate as a function of the manipulation of his phenotype (see Safran et al. 2005). Because social pairs

stay together for a breeding season, this experimental design enabled us to examine changes in a male's paternity solely as a function of the phenotype manipulation, holding constant and thus controlling for features of a male's territory and social mate. We further examined the effect of manipulating ventral coloration and tail streamer length on a male's probability of siring extrapair young in other nests within his colony.

In accordance with current evolutionary theories of multiple signaling (reviewed in Candolin 2003; Hebets and Papaj 2005), experimental enhancement of both signals should elicit the strongest female preference, but enhancement of only one of them is also expected to increase preference to some degree. Such an effect is expected whether multiple signals serve to back up each other (e.g., present redundant information about quality) or whether they serve to convey different messages ("multiple messages"). We therefore predicted, a priori and based on previous correlational data (Vortman et al. 2011), that males whose streamers were elongated or ventral color darkened either alone or in combination with other trait manipulations would garner greater paternity from their social mates and females in neighboring nests compared to the other treatment groups.

Material and Methods

General Methods

We studied a population of barn swallows at two breeding sites in Israel over two consecutive breeding seasons from November 2008 to July 2010. We captured and individually marked swallows with numbered aluminum rings and passive radio-frequency identification (12 mm, 0.095 g) tags. Tags were mounted to the birds by gluing the tags to black 23-mm plastic leg bands. Tag identification was accomplished remotely with a custom-made reader and antenna (Logi Tag Systems, Netanya, Israel). Sites were monitored daily throughout the breeding season, and nests were assigned to breeding pairs. We first allowed a female to settle

Table 1: Average male tail streamer length and ventral coloration chromatic score from the European population (*Hirundo rustica rustica*), east Mediterranean population (*H. r. transitiva*), and Egyptian population (*H. r. savignii*)

Subspecies	Tail streamer length \pm SD (mm; <i>n</i>)	Ventral coloration chromatic score \pm SD (R/G ratio; <i>n</i>)
<i>H. r. rustica</i>	107.66 \pm 8.94 (781) ^a	1.25 \pm .12 (38)
<i>H. r. transitiva</i>	102.7 \pm 8.8 (102)	1.41 \pm .15 (39)
<i>H. r. savignii</i> ^b	90.6 \pm 7.3 (22)	2.16 \pm .14 (19)

^a Data on tail streamer length of the European population was taken from (Møller 1994).

^b All data of *H. r. savignii* was collected from skins at the Bird Group collection of the British Natural History Museum, Tring.

with a mate and lay a complete clutch of eggs. After 7–10 days of incubation, both the male and female attending a given nest were captured, measured, marked, and sampled for DNA. Males were randomly assigned to one of six treatment groups: (1) control (CC), neither tail streamer length nor ventral coloration was altered, but males were measured and held as if they were manipulated; (2) tail streamers shortened (S); (3) ventral coloration enhanced (darkened [D]); (4) tail streamer elongated (E); (5) both ventral coloration enhanced and tail streamers elongated (DE); and (6) ventral coloration enhanced but tail streamers shortened (DS), giving these manipulated males a *savignii*-like appearance (fig. 1). Following the manipulation, we immediately removed the first clutch of eggs, thereby inducing the female into laying a replacement clutch after she had the opportunity to reassess her social mate's quality. DNA samples from each embryo in the first clutch and from each nestling in the replacement brood were used to compare paternity allocation to the same male as a function of changes in signals of male quality by directly analyzing differences in the proportion and number of extrapair young between the first and replacement clutches of males in each treatment group. To avoid asynchrony of the manipulated pairs with the rest of the colony, we collected eggs from all nests in the colony, even if their males were not included in the experimental sample. We captured nearly all males in each colony ($n = 102$), including unmated males or males that were not included in the experiment ($n = 35$), to allow assignment of possible extrapair fathers within the colony. A total of 67 males were assigned randomly among experimental treatments (for details on per treatment group valid n , see table 2).

Ornament Manipulations

A male's natural streamer length was shortened or elongated by 13 mm, the equivalent of 1.5 SD of the variation within the natural population. Tail streamer length was altered following Bro-Jørgensen et al. (2007), where streamers were cut at about 15 mm from the base and a new pair of streamers (obtained from migrating European males caught at a distant roosting site) was attached to the stump. To ensure that our manipulation either increased or decreased the original tail length by 13 mm, the distal portion of the feather was trimmed to the exact length needed for the two treatments with respect to the male's original streamer length. Note that streamers are similar with respect to dull black coloration across barn swallow subspecies, so the new streamer that was attached to the experimental male blended in with the color of his other rectrices (Y. Vortman, personal observation). The 13-mm experimental reduction or elongation of the tail streamer is consistent with the morphology of the short-tailed Egyptian (*Hirundo rustica savignii*) and long-tailed European (*H. r. rustica*) populations, respectively (fig. 1; table 1). Ventral coloration was modified following Safran et al. (2005) by using a nontoxic permanent marker (PrismaColor, light walnut), which enhanced the ventral plumage by an average chromatic amount of 0.45 ± 0.04 (\pm SE) R/G ratio (see "Color Analysis and Color Scoring"), the equivalent to 3 SD of the natural variation and within the range of the very dark Egyptian population (fig. 1; table 1).

Color Analysis and Color Scoring

To measure ventral coloration we photographed the ventral plumage of each individual in the field on capture.

Table 2: Treatment effect on the differences between the first clutch and the replacement brood in within-pair paternity and within-brood number of extrapair young

Treatment group	Valid n	Between-brood difference in paternity proportion			Between-brood difference in within-brood number of extrapair young		
		Estimate \pm SE	t	P	Estimate \pm SE	t	P
CC	7	.083 \pm .118	.7	.58	-.33 \pm .49	-.67	.64
S	6	.033 \pm .126	.26	.8	-.21 \pm .52	-.41	.69
D	8	.083 \pm .112	.74	.58	-.30 \pm .47	-.63	.64
E	6	-.11 \pm .126	-.93	.58	.61 \pm .53	1.17	.51
DE	6	.32 \pm .126	2.56	.05	-1.55 \pm .53	-2.93	.02
DS	6	-.4 \pm .126	-3.22	.02	1.78 \pm .53	3.37	.02

Note: Parameter estimates for treatment effects were tested against the null hypothesis that the difference between the first clutch and the replacement clutch would not significantly differ from 0. SE = standard error based on a pooled estimate of the error variance. The P values were corrected for false discovery rate following Benjamini and Hochberg (1995). Significant values are shown in bold.

We applied digital photography after verifying that the natural ventral plumage and the manipulated ventral plumage had no reflectance peak in the UV range of the spectrum. This was done by using an Ocean Optics USB-4000 spectrometer (range, 200–1,100 nm; Ocean Optics, Dunedin, FL) to measure feathers mounted on cards. Data on variation in color for *H. r. savignii* males were obtained by measuring 22 museum male specimens (Bird Group collection at the Natural History Museum at Tring, UK). We applied digital photography following Stevens et al. (2007), using raw file formats (Nikon Electric Format) and manual white balance (for detailed camera settings, see Vortman et al. 2011). In order to analyze digital images and score color, we used the MATLAB (Mathworks, Natick, MA) tool *Hirundo* (<http://ibis.tau.ac.il/twiki/bin/view/Zoology/Lotem/YoniVortman>; Vortman et al. 2011), developed together with the Signal and Image Processing Lab at Technion, Israel. The lack of UV reflectance peak enabled us to score color using the sRGB color space. Scoring feathers' chromatic elements on the R/G and G/B ratio is consistent with vertebrates' perception of chromatic properties (Hering 1878; Hurvich and Jameson 1957) and with its relative insensitivity to variations in lighting intensity (Kelber et al. 2003). Finally, because in the very dark Egyptian subspecies blue reflectance was too low, we further simplified our color-scoring method using the single R/G ratio following an established method for scoring colors from digital images (Bergman and Beehner 2008).

Paternity Assignment

DNA of adults and nestlings was extracted from blood samples, and DNA of embryos collected from the first clutches was extracted directly from the embryo tissue sample using DNeasy 96 blood and tissue kit (Qiagen, Valencia, CA). To determine whether offspring were sired by their social parent or another male in the population, we amplified seven microsatellite loci (Vortman et al. 2011; see also table A1 [tables A1–A3 available online] for descriptions of each microsatellite, including PCR conditions), creating a powerful test for exclusion of social males in cases of extrapair young within the brood, which was required for our main analysis (second parent exclusion probability = 0.9999). Further, for a secondary analysis, each offspring was assigned to its most likely genetic father and independently to the most likely parent pair, considering the social mother's genotype. Extrapair fathers were identified only when both assignments converged to the same genetic father and only when both the social mother's and father's genotypes were known (for 14 of 96 extrapair young, we were not able to assign an extrapair father). In none of the cases of assigned extrapair fathers did the

parent-pair analysis (offspring–mother–suggested father) yield similar likelihood (LOD) scores to the two most likely fathers (i.e., only the first most likely father had a significant LOD score). Further, we note that while inclusion, or paternity assignment, is error prone, it is not likely to be systematically biased toward a specific manipulation group. All paternity analyses were performed using Cervus, version 3 (Marshall et al. 1998; Kalinowski et al. 2007).

Statistical Analyses

We analyzed the effect of the phenotype manipulation ($n = 6$ treatments) on the difference in (1) proportion of a male's paternity and (2) the number of extrapair young (young not sired by social father) in his own nest between the two successive breeding attempts. To analyze proportional differences in paternity, we calculated the differences in a male's paternity between the two successive broods and used that as the response variable. For the model in which we explored differences in the number of extrapair young within each successive brood, we employed a binomial response variable for paternity outcome (own young/total young in nest). Because of the paired design employed in our experiment, we were able to analyze within-individual changes in paternity by testing whether paternity outcomes varied within a particular treatment (H_0 ; the parameter estimate for differences in these two variables between the first clutch and the replacement clutch are not significantly different from 0). We then corrected the P value controlling for false discovery rate following Benjamini and Hochberg (1995).

Males were randomly assigned to one of the six treatment groups. Across treatments, differences in the number of a male's own young in each breeding attempt (before and after the manipulation) did not deviate from a normal distribution. In contrast, differences in the proportions in number of young between the two breeding attempts were not normally distributed. As the F -tests employed in ANOVA are robust to deviations from the assumption of normality (Sokal and Rohlf 1995), we report these results but also report the results from a nonparametric Kruskal-Wallis analysis.

Levene's test for equality of variances revealed significant differences due to low variance in the S (shorten) steamer treatment group (see fig. 2; mean differences, ~ 0.0). Removal of this group from the data confirmed homogeneity of variances among all other groups both for difference in proportion in within-pair young ($P > .16$) and for the difference in within-pair number of extrapair young ($P > .07$), and rerunning the model without the S group gave equally significant results as the model that contained this S group (see table A2). Statistical tests were carried out with JMP, version 9 (SAS Institute, Cary, NC).

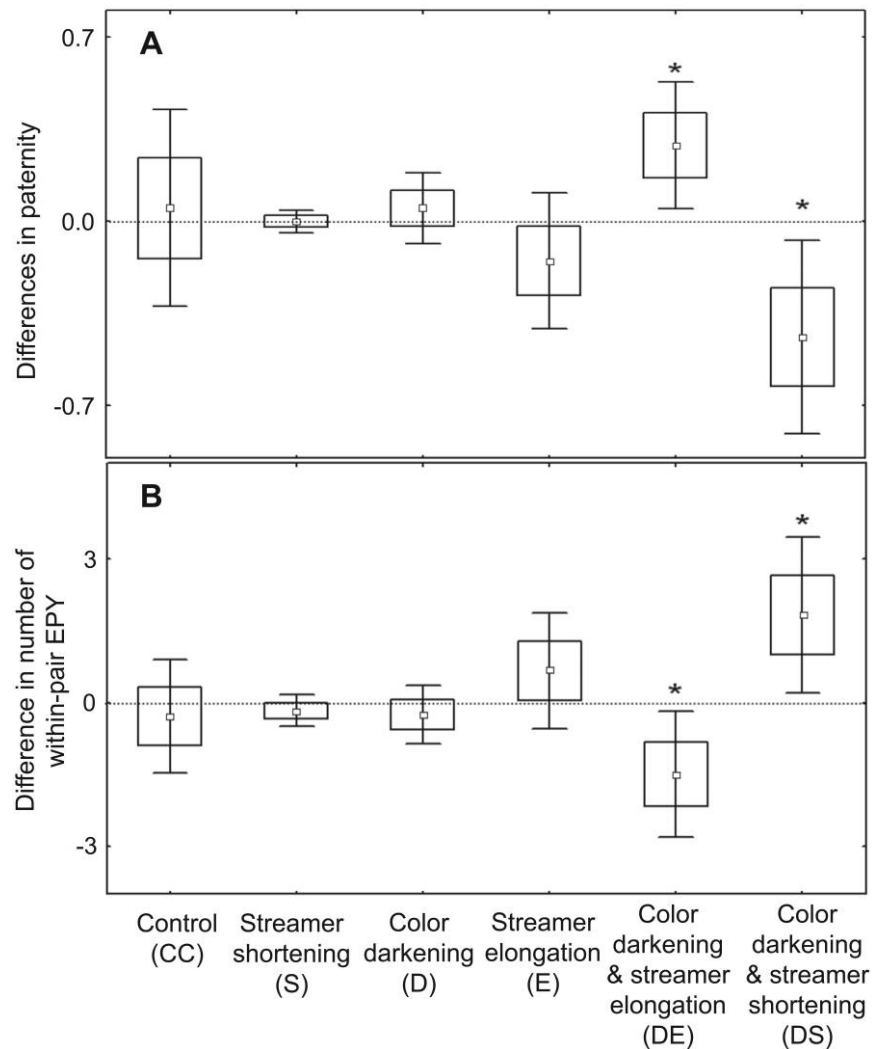


Figure 2: Changes in paternity and number of extrapair young (EPY) within the brood, following the experimental manipulation. *A*, Differences in paternity (proportion of genetic offspring in the replacement brood minus proportion of genetic offspring in the first breeding). *B*, Difference in the number of within-brood EPY (number of EPY in the replacement brood minus number of EPY in the first breeding attempt). Significant changes are marked with an asterisk ($P < .05$) in accordance with table 2. Small squares in the figure represent the means, large squares mean \pm SE, and error bars mean \pm 1.96 SE.

Extrapair Male Assignment

In order to examine the consequences of the phenotype manipulation on siring extrapair young in other nests, we first assigned the extrapair young in each clutch (first clutch or replacement brood) to their extrapair father (see “Paternity Assignment”). We then analyzed whether extrapair young in these focal experimental nests were sired by males that were included in one of the six treatment groups and, importantly, whether these sires’ phenotype was manipulated before or after the first lay date of the extrapair offspring’s clutch. Accordingly, for each male we could score the number of acquired extrapair mates and

extrapair young before or after the manipulation. Thus, following the main results, we could examine whether the increase in within-pair paternity found in males of the DE group was also indicated by an increase in their number of extrapair mates (using a one-tailed Mann-Whitney U -test to compare them with all other males).

Results

There were no significant differences in males’ initial (pre-manipulation) ventral coloration ($F = 0.55$, $df = 5, 33$, $P > .7$), clutch initiation date of the first breeding attempt

($F = 1.4$, $df = 5, 33$, $P > .24$), or initial (premanipulation) tail streamer length ($F = 2.2$, $df = 5, 33$, $P > .08$) between the six treatment groups. Furthermore, none of the above (original ventral color and streamer length or clutch initiation date before the phenotype manipulation) had any effect on the dependent variable in our main analysis (i.e., differences in within-pair paternity or within-pair number of extrapair young before and after the manipulation; $P > .3$ in all cases). We confirmed that there were no significant differences between treatment groups in a male's paternity (own young/total young in nest) at the start of the experiment before the phenotype manipulations were applied (mixed model with binomial response variable: $F = 3.32$, $df = 1, 5$, $P > .40$; see table 3 for within-group details) and in a male's within-brood number of extrapair young (i.e., number of young within the brood that are not sired by the focal male; $F = 1.87$, $df = 5, 33$, $P > .12$; table 3). In contrast, we found a significant effect of treatment on the differences in within-pair paternity (Kruskal-Wallis: $\chi^2 = 13.3$, $df = 5$, $P < .02$; fig. 2A) and on the change in within-brood number of extrapair young ($\chi^2 = 12.0$, $df = 5$, $P < .03$; data available in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.g8n63>; Vortman et al. 2013).

The pattern of this effect was surprising: only males in the DE group whose ventral color was darkened and streamers were elongated received a significant increase in paternity (DE; see fig. 2; table 2), whereas males whose ventral coloration was enhanced but tail streamers were shortened (*savignii*-like males) had significant paternity losses in their replacement broods (DS; see fig. 2; table 2). No significant change in paternity (or in the number of extrapair young at the nest) occurred for males who had only their tail streamers or only their ventral coloration manipulated (fig. 2; table 2, groups E, S, and D).

Yet another analytical approach to these same results is to apply a statistical model with ventral coloration and tail length treatments as fixed factors in a two-by-three design (i.e., two treatments \times reduced, no change, enhanced), which allows an explicit test of the interaction between

them. This analysis nicely illustrates that each treatment alone has no significant effect on the change in within-pair paternity or on the change in the number of EPY but that the interaction between them is statistically significant (see table 4). The fact that only a combined enhancement of both signals increases male success (the positive effect of the DE group) is also suggested when examining the manipulation effect beyond the social brood. For males of the DE treatment group, the difference in the number of extrapair mates (after the manipulation minus before the manipulation) was higher than for all other males (Mann-Whitney U -test: $x^2 = 0.66$, $n_1 = 3$, $n_2 = 21$; one-tailed probability: $P = .033$; see appendix for a detailed within-individual comparison; table A3).

Discussion

Considered in concert, these experimental results confirm that both tail streamers and ventral coloration serve as sexual signals in *Hirundo rustica transitiva* and, further, that the exaggeration of both signals simultaneously is required to increase female preference for social or extrapair mates. The finding that tail elongation or shortening alone (E and S groups) had no effect on male within-pair paternity or on his probability of siring extrapair young is in notable contrast with studies of the European population, where similar tail streamer elongation and reduction have positive and negative effects, respectively, on female preference (Møller 1988; Saino et al. 1997). Similarly, the lack of increase in paternity following color enhancement alone (D group) is in contrast with the findings from a previous study of the North American population, where the same experimental darkening had a strong positive effect on male within-pair paternity (Safran et al. 2005). These results provide a unique example of how different patterns of mate selection on the same set of traits among closely related populations can lead to population-level phenotype divergence.

The lack of an effect in all our manipulations of a single trait (E, S, and D groups) and the highly significant neg-

Table 3: Means and standard errors of within-pair paternity and within-brood number of extrapair young for the different treatment groups before and after the manipulations were conducted

	Treatment group					
	CC	S	D	E	DE	DS
Within-pair paternity:						
Before manipulation	.68 \pm .12	.9 \pm .06	.88 \pm .07	.85 \pm .08	.6 \pm .15	.95 \pm .04
After manipulation	.78 \pm .144	.9 \pm .06	.9 \pm .04	.7 \pm .14	.89 \pm .07	.51 \pm .06
Within-brood number of extrapair young:						
Before manipulation	1.14 \pm .5	.5 \pm .34	.5 \pm .32	.6 \pm .33	2 \pm .85	.16 \pm .16
After manipulation	.85 \pm .46	.33 \pm .21	.25 \pm .16	1.33 \pm .66	.5 \pm .34	2 \pm .77

Note: See statistical analysis in table 2.

Table 4: Separate effects of ventral coloration treatment, tail streamer length treatment, and their interaction on the differences between the first clutch and the replacement brood in within-pair paternity and within-brood number of extrapair young

Treatment	Between-brood difference in paternity proportion			Between-brood difference in within-brood number of extrapair young		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Whole model	3.1	5, 33	.02	3.675	5, 33	.009
Ventral coloration	0	1	1	.009	1	.92
Tail streamer length	2.77	2	.076	2.8	2	.075
Ventral coloration × tail streamer length	4.98	2	.012	6.38	2	.004

Note: Significant *P* values are shown in bold. df = degrees of freedom.

ative effect of the DS treatment group on within-pair paternity was unexpected. The possibility that our results from single-trait manipulation treatments is due to low statistical power seems unlikely because none of these treatment groups shows a clear trend. If anything, the nonsignificant effect of tail elongation alone (E group) is in the opposite direction than expected (see fig. 2). More importantly, low statistical power cannot explain the highly significant negative effect of the DS treatment.

We cannot tease apart whether tail streamer length and ventral coloration serve to convey redundant overall quality or multiple messages. While both are related to feather growth and molt cycle, each may reveal different aspects of male quality. Long tail streamers bear an aerodynamic cost (Møller and de Lope 1994; Evans 1998; Bro-Jørgensen et al. 2007), whereas dark ventral coloration seems to bear a physiological cost (Safran et al. 2008; Galvan and Møller 2011). However, based on either the “redundant message” or “multiple messages” models of multiple signaling, theory predicts that manipulations of a single trait should have had an effect on paternity outcomes (e.g., Hebets and Papaj 2005). Following this logic, we predicted that males whose streamers were shortened and ventral coloration was enhanced (DS) were expected to do better, not worse, than males who had only their streamers shortened (S; see fig. 2). Yet, when considering the unique position of *H. r. transitiva* within the subspecies clade (fig. 1), these puzzling results are intuitive if one considers that multiple signals also serve to discriminate between conspecific and heterospecific males. In light of this potential explanation, the observed negative response of *transitiva* females to the DS treatment is predicted if there is selection to avoid Egyptian (*H. r. savignii*) males whose phenotype matches males in this treatment group (see fig. 1; table 1). For technical reasons we could not lighten ventral coloration to create a comparable local model of high-quality European (*H. r. rustica*) males, but the lack of any response to tail elongation alone (E group; fig. 2) is also consistent with this idea. By relying on strong expression of both

long tail streamers and dark ventral coloration, *transitiva* females can discriminate against high-quality non-*transitiva* males from the European population (that have long streamers but relatively pale ventral coloration). We cannot determine which of the two, sexual selection or discrimination, was the initial force selecting for this unique pattern of female preference in our population. However, we suggest that the current behavior allows females to choose high-quality and locally adapted males.

Using a combination of signals to discriminate between high-quality local and foreign males would make adaptive sense only when there are costs associated with hybridization and as long as the signals used to identify local males are heritable (thus reflecting genetic origin). Indeed, molecular analysis (Dor et al. 2010, 2012) and detailed studies of the biogeographical history of the region (Goodfriend and Magaritz 1988; Robinson et al. 2006) suggest recent divergence and secondary contact events between closely related subspecies of barn swallows (*savignii* to the south and *rustica* to the north). Marked behavioral differences in migratory and breeding behavior (Møller 1994) suggest that specific ecological adaptations have evolved in each population, which may increase the likelihood of hybrid inferiority. Moreover, based on the results that males with both dark ventral plumage and long streamers are favored by females, one can infer that mating with a male that lacks one of these signals (e.g., Egyptian or European) would almost certainly reduce the relative sexual attractiveness of her future male offspring and should be selected against. This scenario is especially likely due to the fact that tail streamer length and ventral coloration are significantly heritable in our barn swallow population (Y. Vortman, T. Reiner Brodetzki, R. J. Safran, R. Dor, and A. Lotem, unpublished manuscript).

Broader Implications of These Results

The observed female preference of multiple sexual signals in the Israeli population may reduce gene flow from ad-

adjacent populations and maintain the intermediate population multiple-ornament morphology (fig. 1). If so, mate selection is shaping the same set of traits in different ways in closely related populations; thus, phenotype novelty is not involved in population differentiation and, rather, variation in the strength or direction of selection on multiple aspects of similar features of phenotype are of importance. If females' avoidance of males who express only an exaggeration of one of these phenotypes rather than both (e.g., in the case of this study, color in *H. r. savignii* and tail streamer length in *H. r. rustica*) becomes more pronounced, further reduction in gene flow and complete divergence may be possible in what may be viewed as a reproductive character displacement–like process. While reproductive character displacement is usually perceived as a process that is based on different traits or sexual signals that uniquely characterize each population (Saetre et al. 1997; Servedio 2004), our results lead us to suggest that it can also be based on a mixture of traits that can distinguish an intermediate or a hybrid-origin population from its adjacent populations. This idea is in line with increasing recognition that hybridization, gene flow, and introgression can promote speciation (Mavarez et al. 2006; Gray and McKinnon 2007; Mallet 2007; Brelsford 2011; Hermansen et al. 2011). Such processes are likely to create closely related populations that share similar features of signal traits but can still be distinguished from one another by different combinations of these signal traits. Accordingly, we predict that the use of multiple sexual signals, in general, may be more frequent in intermediate or contact zone populations. Further, we suggest that the discovered link between multiple sexual signals and species richness (Seddon et al. 2008) may also be attributed to the fact that conditioning mate choice on multiple sexual signals may also improve female discrimination between conspecific and heterospecific males, thus facilitating prezygotic reproductive isolation between diverging populations.

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