

Science

25 January 2008 | \$10



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 AAAS

25 January 2008

Science

Vol. 319 | No. 5862 | Pages 369-532

www.sciencemag.org

Adaptive Plasticity in Female Mate Choice Dampens Sexual Selection on Male Ornaments in the Lark Bunting

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Theory on the evolution of ornamental male traits by sexual selection assumes consistency in selection over time. Temporal variation in female choice could dampen sexual selection, but scant information exists on the degree to which individual female preferences are flexible. Here we show that in lark buntings sexual selection on male traits varied dramatically across years and, in some cases, exhibited reversals in the direction of selection for a single trait. We show that these shifts are probably because of flexibility in mate choice by individual females and that they parallel shifts in the male traits that predict female reproductive success in a given year. Plasticity in choice and concomitant reversals in mating patterns across time may weaken the strength of sexual selection and could maintain genetic variation underlying multiple sexual ornaments.

Sexual selection for exaggerated secondary sexual traits arises from two different mechanisms that result in correlations between male traits and mating success—male-male competition for access to females and female mate choice (1, 2). These mechanisms can be difficult to disentangle (3), but direct female choice for extravagant male traits has been shown in many taxa (2). Ornament evolution via female choice requires that females prefer an extreme expression of a male trait, that trait preferences are concordant among females in a population, and that these preferences are fairly consistent across time (4, 5). Changes in the ecological or social environment could favor flexibility in female preferences (6, 7), but information about the temporal consistency of female choice is currently lacking (6, 8). Plasticity in female preferences could have major effects on the strength and outcome of sexual selection (9) and, potentially, could slow trait exaggeration (10).

We investigated the dynamics of pairing patterns and mate choice in the lark bunting, *Calamospiza melanocorys*, a migratory songbird breeding on the short-grass prairie of Colorado. Sexual selection is potentially strong in lark buntings, because many males fail to attract a social mate [~45% of territorial males (11)] because of a male-biased breeding sex ratio coupled with social monogamy and because extra-pair paternity is common [25% of young and 47% of broods (11)], but variable among males. To assess the dynamics of sexual selection, we studied five independent male plumage traits and three measures of size (Fig. 1)—body color, proportion of black versus brown feathers separately on the rump and the rest of the body, wing patch size, wing patch color, body size, beak size, and residual mass (12). To examine sexual selection on these male traits,

we assessed both the social pairing success of color-banded males in each breeding season, as well as their total annual fitness, using micro-satellite parentage analysis (12).

In territorial birds, it can be difficult to distinguish between direct female choice for male traits and female choice for territory features correlated with male traits (i.e. male dominance badges). Male lark buntings, however, are only weakly territorial until mate acquisition, at which time the territory is no longer defended nor respected by other males

[new males begin displaying on the former territory (11)]. Display territories are not used for feeding by either males or females (including offspring feeding), but females nest near or on the display territory, so we quantified territory quality as the density of woody shrubs available for use as nest cover (12).

In each of the 5 years of this study, plumage or size characteristics of males were associated with total male fitness [number of within- and extra-pair fledglings sired (12) (Fig. 2)], which indicated significant potential for sexual selection on those male traits. However, a significant effect of year on the traits that correlate with male fitness indicated that the specific traits under sexual selection varied among years [according to a generalized linear model (GLM): full model $F_{3,380} = 5.19$, $P = 0.001$; effect of year: $F_{4,379} = 7.52$, $P < 0.001$ (12)]. Examination of these patterns revealed dramatic changes across years in the suites of traits that predicted male fitness, and moreover, no two years showed similar patterns of male traits associated with fitness (Fig. 2).

We observed two distinct types of change in the pattern of selection on male traits across years. First, some male traits were under strong selection in some years, but showed very weak selection or no selection in other years [beak size and rank body color (Fig. 2)]. Cubic splines illustrate the specific form and intensity of phenotypic selection on male traits each year (12, 13) and demonstrate the occurrence of this on-off pattern of selection

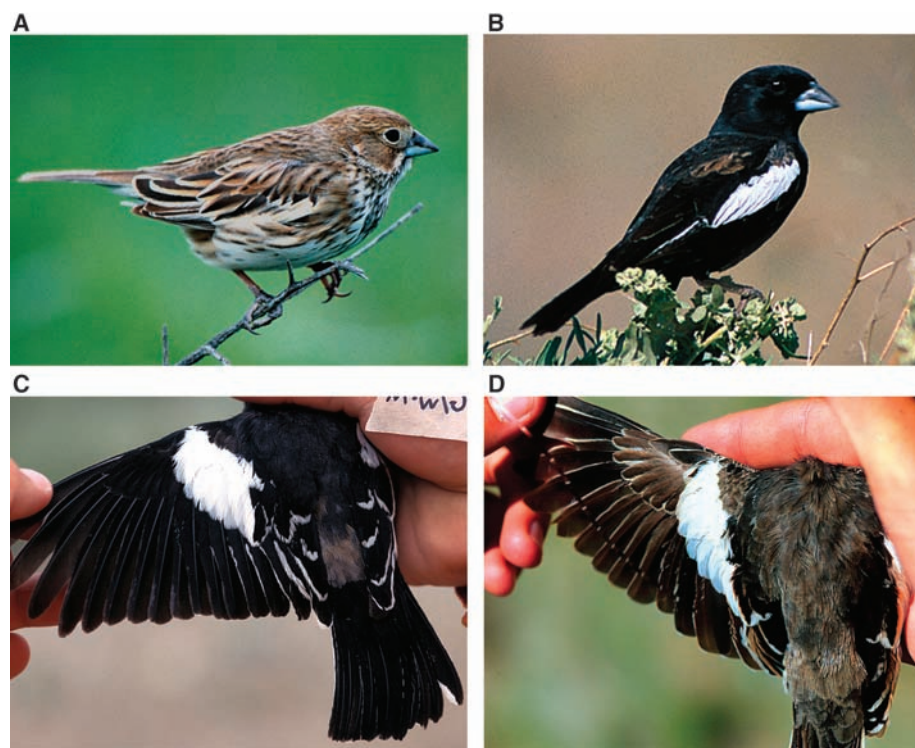


Fig. 1. Plumage traits of lark buntings. (A) Females are brown with dull white wing patches, whereas (B) males are generally black, but often have patches of brown feathers that vary in size and distribution (note patch above wing). The conspicuous white wing patches vary in both size and color among males and are both larger and brighter than those of females. There is considerable variation in color between (C) black males and (D) gray ones. Likewise, the proportion of dark versus brown feathers varies among males on both the body [(C) versus (D)] and rump (C) relative to other body parts.

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for several traits [colored lines (Fig. 3)]. Similar effects have been shown in other species (14, 15). Second, three traits—body size and the percentage of black feathers on both the rump and the rest of the body—showed a positive association with male fitness in 1 year and a negative association in another year. These reversals from positive to negative selection gradients were significant (GLM: year \times body size $F_{4,379} = 3.56$, $P = 0.007$; year \times rump% $F_{4,379} = 3.25$, $P = 0.012$; year \times body% $F_{4,379} = 3.08$, $P = 0.016$) and indicate that dramatic shifts in selection occur across years (Fig. 2) and colored lines in (Fig. 3)]. The reversals in selection on male traits we document here provide a sexual selection parallel to oscillating natural selection described for Darwin's finches (16).

To understand the underlying cause of variable selection on male traits, we investigated a key component of male fitness: acquisition of a social mate. The general pattern of selection on male traits through mate acquisition (Fig. 4) was similar to overall selection on male traits (Fig. 2)—there was a significant association between male traits and mate acquisition, but the traits of successful males varied across years [GLM: full model $F_{3,380} = 10.22$, $P < 0.001$; effect of year: $F_{4,377} = 4.13$, $P = 0.002$; (12)]. Some traits were under selection because of mate acquisition in some years but not others (percentage black feathers on the body, beak size, and residual mass). Two traits—wing patch size and rank body color—showed changes in the direction of selection across years (GLM: year \times wing patch size $F_{4,415} = 3.24$, $P = 0.012$; year \times rank color $F_{4,415} = 3.29$, $P = 0.011$). A randomization test (12) indicated a close correspondence between the traits under selection because of total fitness and those under selection because of mate acquisition [Fig. 2 versus 4, $P < 0.001$, (12)]. The striking similarity in patterns of selection on male traits through total male fitness and through mate acquisition occurs because the majority of offspring are sired within the social pair [75% (11)] and suggests that across-year variation in social mating success is a major driver of variable selection on male traits.

Several observations suggest that temporal fluctuations in social mating patterns are more likely to be because of changes in female choice for male traits than changes in traits that influence competition among males for high-quality territories that females might choose. First, territory quality (12) was never associated with mate acquisition [i.e., was not selected by Akaike's Information Criterion (AIC) models for all years combined or for any individual year; partial $P > 0.2$ in all cases] and was therefore removed from all selection models. Second, we determined whether traits previously found to be important to male-male competition (11) differed across years in their importance as dominance signals. We did this using both observational and experimental methods, but in no case did the male traits associated with social dominance change across years in a manner that could explain the dynamic patterns of mate acquisition we describe here

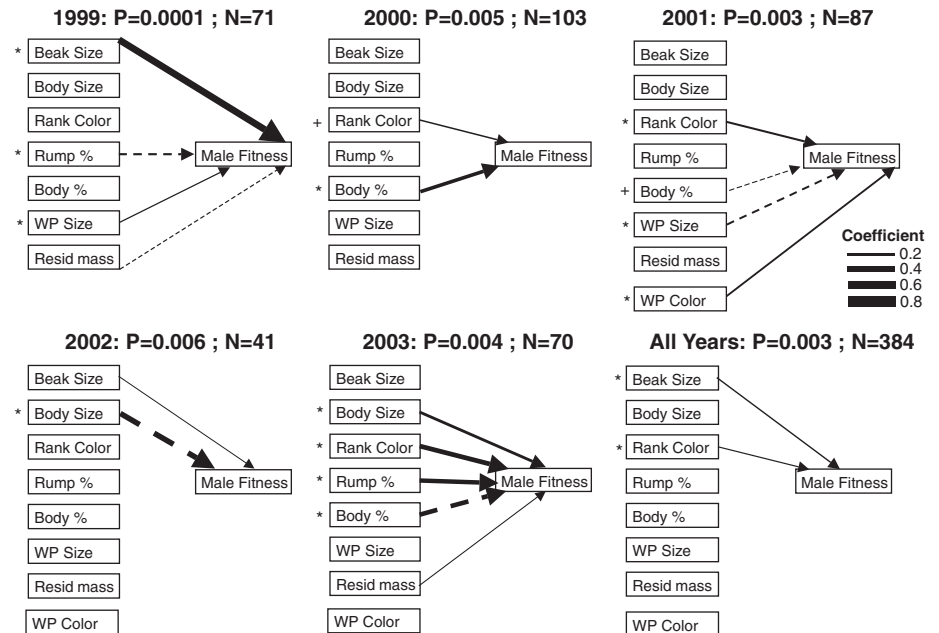


Fig. 2. Male traits associated with total male fitness (number of sired within and extra-pair chicks that fledged) show dramatic across-year variation. Year, P value, and sample size from the final GLM (12) are above each diagram. Thickness of arrows between traits and fitness indicate the partial correlation coefficient (selection gradient). Dashed and solid arrows are negative and positive relationships, respectively. Traits include three morphometric measures (beak size, body size, and residual mass) and five plumage measures: body feather color (rank color), percentage black feathers on the rump (rump%) and the rest of the body (body%), wing patch size (WP size), and wing patch color (WP color). Partial effect in GLMs designated as $*P < 0.05$, $+P < 0.1$.

(12). Furthermore, the level of male aggression in response to a standardized stimulus did not differ across years, which suggested that the intensity of male-male competition did not vary across years (12).

Two lines of evidence indicated that population-level changes in female mate choice across years were because of plasticity in individual female preferences across years rather than age-related or other demographic shifts (12). Females that were observed in 2 or more years and that did not stay with the same social mate (12) were used to estimate the degree of individual consistency [repeatability, r_t (17)] in mate choice (19 females whose mates were also caught: 17 in 2, 1 in 3, and 1 in 4 years). Mate choice (traits of her chosen mates) was not significantly repeatable across time (repeatability: all traits $df = 2.2, 19$, $r_t < 0.18$; $P > 0.2$), which suggests substantial plasticity of female choice. Indeed, most females showed considerable change in the traits of their mates across years (fig. S1). Changes in the mate choice decisions of individual females will only sum to population-level dynamic sexual selection if most females show similar patterns of plasticity and response, a pattern that implicates the influence of external factors such as the social or ecological environment (18, 19).

We investigated the possibility that plasticity in choice is adaptive because it allows a female to choose a male or territory character that will maximize her fitness in each year. This hypothesis predicts that females target male or territory

characters that predict fitness benefits to them in a given year, that different traits serve as fitness indicators in different years, and that changes in female preference across years correspond non-randomly with the changes in fitness-indicator traits. In most years, one or more male traits were correlated with nesting success (a measure of female fitness, fig. S2) making them potential indicator traits, and different traits were predictors of nesting success in different years (fig. S2). Randomization tests determined that there were significantly more matches than expected by chance between the traits of males chosen by females and the male traits associated with nesting success within years [four of seven male traits correlated with nesting success were also preferred by females, $n = 76$ total trait-year possibilities, $P = 0.005$; see (12)]. This result suggests that flexible female choice enables females to track temporal variation in the traits that predict enhanced nesting success. However, it remains unknown whether the specific traits we measured or correlates of those traits are the actual targets of female choice (20).

Sexual selection by female choice requires that the cumulative effects of female preferences be fairly consistent over time (1, 2), and models of sexual selection with consistent choice predict extreme exaggeration of male traits (4, 5). However, if female choice varies across years, phenotypic selection for male trait exaggeration could be dramatically reduced or even eliminated, as has been suggested for temporal variation in natural selection (21, 22). We investigated the

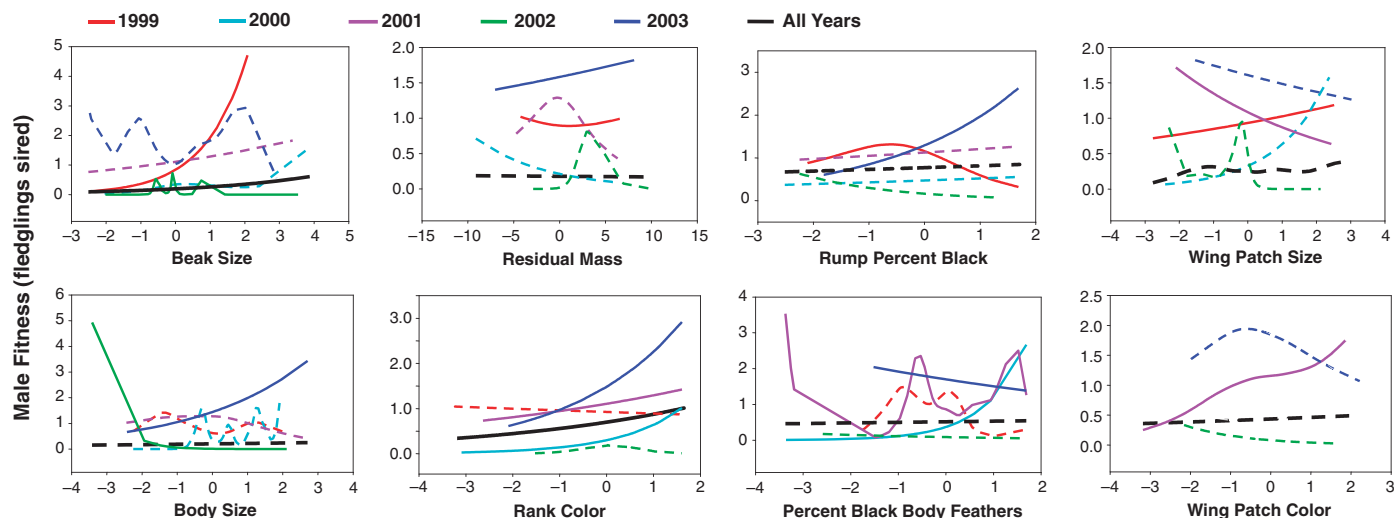


Fig. 3. Cubic splines reveal the dynamic nature of selection on male traits because of male fitness (number of fledglings sired within pair and extra-pair) across years. Colored splines represent selection on male traits in a given year; black splines represent selection in all years combined. Wing patch size and the percentage of black feathers on both the rump and rest of the body show marked reversals in the direction of selection,

whereas rank body color and beak size show consistent positive relationships in all years. Solid lines depict traits that entered into selection models [models and *P* values in (Fig. 2); see (12)] for that year (or all years), whereas dashed lines were not significant. Only rank color and beak size show significant selection in the analysis using data from all years combined.

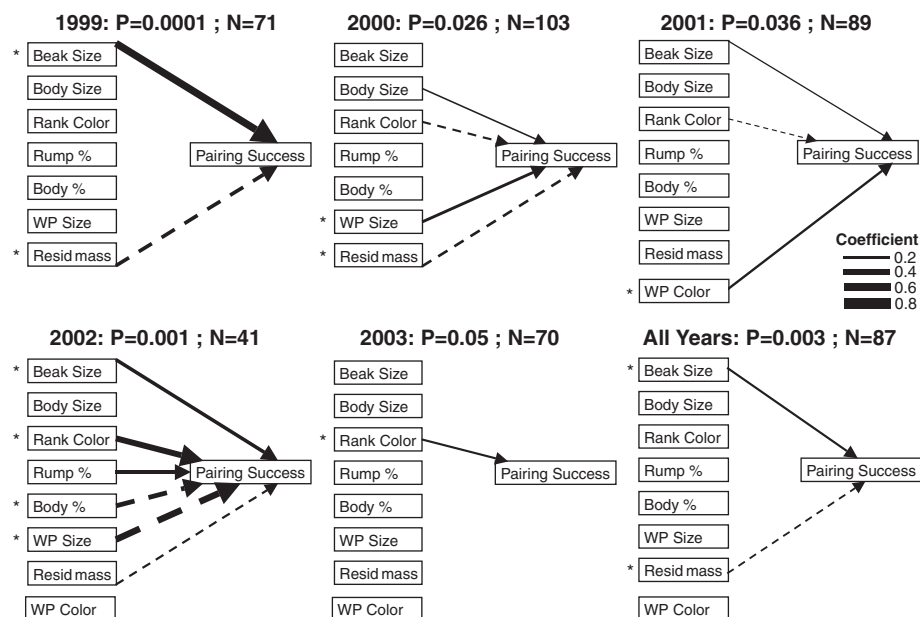


Fig. 4. Male traits associated with pairing success (mated or not mated) differ dramatically across years, presented as in (Fig. 2). Traits include three morphometric measures (beak size, body size, and residual mass) and five plumage measures: body feather color (rank color), percent black feathers on the rump (rump%) and rest of the body (body%), wing patch size (WP size), and wing patch color (WP color). Partial effect in GLMs designated as **P* < 0.05.

impact of temporal scale on phenotypic selection by comparing analyses from individual years to an analysis of data combined across years [e.g. (Fig. 2)]. For most traits, phenotypic selection of all years combined was weak and only two traits—beak size and rank body color—were under significant selection as determined by overall male fitness patterns (Fig. 2). A similar pattern can be seen in selection on male traits through female choice of a social mate (Fig. 4). The striking effect that temporal scale has on the intensity and pattern

of sexual selection can be seen by comparing selection splines on individual male traits from each year separately with those from all years pooled [colored versus black lines (Fig. 3 and fig. S3)]. By examining years separately, a very different picture of selection on male traits and female mate choice emerges from the standard approach obtained by summing across years. In lark buntings, selection on male traits is often strong within years, dynamic across years, but weak or nonexistent over a 5-year period.

Which time scale is the most appropriate for understanding sexual selection? We suggest that a longer time frame is most appropriate for predicting male trait evolution because annual male fitness measures used to estimate short-term sexual selection are unreliable when selection fluctuates across years. Short-term studies may thus prove insufficient for correctly understanding the strength and direction of sexual selection in species with variable sexual selection and flexible mate choice. In contrast, a short time frame is essential for understanding selection on female mating preferences. Only by examining female choice patterns within years was it possible to discover flexibility in choice and show the adaptive benefits of flexible choice to females.

Plasticity in female preferences has several implications for the process of sexual selection, the evolutionary dynamics of exaggerated traits, and the evolution of female choice itself. As a parallel to natural selection under variable environmental conditions, flexible female choice and dynamic sexual selection could make assessment of selection and predictions for male trait evolution unreliable (22) and may also provide a mechanism for the preservation of genetic variation in traits (23). In addition, most models of sexual selection assume that the evolution of exaggerated traits is stabilized by the costs that exaggerated traits incur (5, 24). In taxa with flexible female preferences, however, choice itself may result in stabilizing selection on exaggerated traits. Finally, flexible female choice may provide an explanation for the evolution and maintenance of multiple male ornaments. Flexible choice should allow a female either to choose a mate that best complements her needs in a given year, where such needs change, or to track the best signals

when signal content changes over years because of changes in the physical or social environment. When females vary their preferences across years, the expression of more than one quality by a male would give him a mating advantage across breeding seasons by providing broad appeal under unpredictable breeding conditions. Under this scenario, selection should favor the evolution of new indicator traits that prove useful to females in some years and maintain existing multiple signals despite countervailing costs. Variation in female preferences may provide explanations for what is currently considered noise [e.g., unexplained variance, (8); low repeatability, (25, 26)] in female choice. Testing for and incorporating temporal flexibility in female choice has the potential to greatly alter our perspectives on the process of sexual selection and trait exaggeration.

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27. We thank J. Bradbury, R. Calsbeek, N. Dominy, R. Montgomerie, and K. Wasson for comments on a draft; B. Sinervo, W. Koenig, A. Ritter, E. Svensson, E. Ferree, D. Shizuka, J. Barna, and T. Price for feedback; J. Barna and volunteers for assisting with data collection; K. Mesa, G. Pogson, M. Burford, C. Fernandez, and J. Thompson for advice on genetics work completed at the University of California, Santa Cruz (UCSC) Molecular Ecology and Evolutionary Genetics (MEEG) facility, and the Pawnee National Grassland and Jones Family for use of their land. Funded by UCSC–Special Research Grant, National Geographic Society, NSF–Doctoral Dissertation Improvement Grant (DDIG) (IBN-0309215), NSF–Graduate Assistantships in Areas of National Need (GAANN), American Museum of Natural History Chapman Fund, Sigma Xi, and the American Ornithologists' Union.

Supporting Online Material

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Materials and Methods

SOM Text
Figs. S1 to S4
References

13 August 2007; accepted 14 December 2007
10.1126/science.1149167

Control of Genic DNA Methylation by a jmjC Domain–Containing Protein in *Arabidopsis thaliana*

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Differential cytosine methylation of repeats and genes is important for coordination of genome stability and proper gene expression. Through genetic screen of mutants showing ectopic cytosine methylation in a genic region, we identified a jmjC-domain gene, *IBM1* (*increase in bonsai methylation 1*), in *Arabidopsis thaliana*. In addition to the ectopic cytosine methylation, the *ibm1* mutations induced a variety of developmental phenotypes, which depend on methylation of histone H3 at lysine 9. Paradoxically, the developmental phenotypes of the *ibm1* were enhanced by the mutation in the chromatin-remodeling gene *DDM1* (*decrease in DNA methylation 1*), which is necessary for keeping methylation and silencing of repeated heterochromatin loci. Our results demonstrate the importance of chromatin remodeling and histone modifications in the differential epigenetic control of repeats and genes.

Genomes of vertebrates and plants contain a substantial proportion of transposons and repeats (1). These potentially deleterious sequences are cytosine-methylated and inactivated (2, 3) to form heterochromatin (4, 5). Methylated heterochromatin, especially when dispersed within gene-rich regions, has the potential to spread by self-reinforcing mechanisms (6, 7) to flanking cellular genes and disrupt their expression. Mechanisms that confine the methylated regions remain enigmatic, despite their importance in maintaining the integrity of large genomes with a high proportion of

dispersed transposons. Here, we identify a new pathway that excludes cytosine methylation from genic regions by histone modification and chromatin remodeling, thus ensuring proper plant development.

In the flowering plant *Arabidopsis thaliana*, and in plants in general, cytosine methylation is found in both CG and non-CG contexts. In *Arabidopsis*, methylation at CG sites is maintained by the DNA methyltransferase MET1, whereas methylation at non-CG sites requires the DNA methyltransferase CMT3 (8–12). Non-CG methylation is also controlled by methylation of histone H3 at lysine 9 (H3mK9) and by the RNA interference (RNAi) machinery (13–16). DDM1, a chromatin-remodeling adenosine triphosphatase, is involved in maintenance of both CG and non-CG methylation (17–19). Mutations in *MET1* and

DDM1 also result in a variety of developmental abnormalities by inducing heritable changes in other loci (8–10, 20, 21). One of the *ddm1*-induced abnormalities, called *bonsai* (*bns*), is caused by epigenetic silencing of a gene encoding a homolog of a cell cycle regulator, *APC13* (22). The silencing of this gene, *BONSAI* (*BNS*), is associated with spreading of methylated heterochromatin from a flanking LINE retroelement (22). This LINE functions as methylated heterochromatin, which has a potential to spread to the flanking *BNS* gene (Fig. 1A).

To explore the mechanisms that exclude genic cytosine methylation in wild-type plants, we used methylation-sensitive restriction enzymes to screen a mutagenized population for individuals with *ibm* (*increase in BONSAI methylation*) phenotype (23). One of them, *ibm1*, is described in this report. The *IBM1* gene (*At3g07610*) was identified by a map-based approach (23). The original *ibm1-1* mutant has a base substitution causing an amino acid substitution (Gly⁶⁷² → Glu). We subsequently tested three additional *ibm1* alleles carrying T-DNA insertions and verified that these independent alleles also caused DNA hypermethylation of the *BNS* gene (Fig. 1B).

The *BNS* sequence was hypermethylated in the first generation in which the *ibm1* mutant allele became homozygous (Fig. 1B). This feature was different from the *BNS* hypermethylation in the *ddm1* mutant, which is slow and detectable only after several generations of self-pollination in the mutant background (22). Bisulfite sequencing revealed that cytosine methylation occurred at the *BNS* gene in *ibm1* and that non-CG sites are the main targets of the *BNS* methylation (Fig. 1C and table S1). Unlike *ddm1*, the *ibm1* mutation did not affect methylation in repeat

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Supporting Online Material

Materials and Methods:

Study Population: We observed individually marked birds from 1999-2003 on a 48 hectare study site (expanded to 80 hectares in 2002 and 2003) on the Pawnee National Grassland, Colorado USA. Birds were trapped off territory at baited feeding stations throughout the season and all individuals were measured (see below) and marked with unique color band combinations for field identification. Blood samples (~20µl) for paternity analyses were taken from all trapped individuals and from nestlings just prior to fledging and stored in lysis buffer (*S1*). Males were observed at least every other day for 10 minutes from when they first established their display territories to when their females completed laying their clutch of eggs to determine residency status, territory location, pairing status, and the outcome of aggressive social interactions. Nests were monitored daily to determine the number of chicks each pair successfully fledged.

Social pairing status was determined either by male association with a nest or using behavioral indices. Most males (207/233) were confirmed as paired by identifying them when they were flushed from their nest, either during incubation (*S2*, *S3*) or when seen feeding chicks, but in most cases they had previously been identified as a paired male based on behavioral criteria (see below) In no case did more than one male incubate or feed at a single nest. In some cases, males were considered paired with a female if three or more of the following criteria were met: 1) they were seen closely associating with a female alone on several consecutive days, 2) the pair conducted characteristic behaviors when alone (unforced copulations, post-copulatory display, following behavior; *S2*), 3) the male no longer displayed on his former territory (see below), and in some cases 4) the male was seen clearly defending a female during neighbor harassment. Many males that fit these criteria were also subsequently associated with a nest, thereby validating our method of assessing pair status from behavioral data.

Territoriality in lark buntings differs from the classic view of avian territoriality. Early in the breeding season male buntings vigorously defend territories from which they perform aerial display flights. Display territory boundaries are often fluid and can change dramatically over time (within days). Once paired with a social mate, males completely cease territory defense and the pair often roams far from the male's former display territory. Prior to clutch completion, many mated pairs are often closely accompanied by small groups of males seeking extra-pair copulations (extra-pair copulations appear to be forced; *S4*). Paired males tolerate these extra-pair attending males and overt aggression is rare. Throughout the entire breeding season buntings forage over broad areas and most food is obtained away from the space initially defended as a display territory. This behavior made it possible for us to capture birds at feeding stations throughout the study area, and many birds were captured long distances from their territories or nests. Females build their nests in the general vicinity of male display territories and the one feature of male territories that could potentially matter to females is the quality of the vegetation for nest cover. A previous study of lark buntings indicated that microgeographic variation in nesting cover — specifically the amount of shade provided to the ground nests — may be critical to female settlement decisions and to nesting success (*S5*) and other studies suggest that vegetation cover is an important nesting criteria for lark buntings (in *S2*) and contributes to nesting success (*S3*).

Serial monogamy across years was rare, largely because it was rare for both members of a pair to return in a subsequent year. In our analysis of repeatability of mate choice, we included only the 19 returning females who paired with different males across the two years being compared (22 transitions) but omitted the two cases where a female paired with the same male across consecutive years. Inclusion of these cases does not alter the results.

Vegetation Sampling and Territory Quality:

To investigate spatial aspects of territoriality, nest site selection and social interactions, we divided the study area into one-hectare quadrats with conspicuous location markers visible from at least 100 meters. To quantify nest vegetation cover, we characterized the vegetation on a fine scale (200 point samples/hectare; 9,600 total samples) over our entire 48-hectare main study plot. In each one hectare quadrat, we ran four evenly spaced 100 meter transects and identified the species of plant that touched the transect line at each 2 meter interval. We then tallied the total number of individual plants of the four woody perennial species that together accounted for nest plants of 85% of the 430 nests assessed: Four-winged Saltbush (*Atriplex spp.*), Spreading Buckwheat (*Eriogonum effusum*), Gray Rabbitbush (*Chrysothamnus nauseosus*) and Broom Snakeweed (*Gutierrezia sarothrae*). Females do not always nest within the boundaries of the male's display territory, but invariably nest in the general vicinity. We therefore used the density of these nest plant species in the one-hectare quadrat that contained the approximate center of the male's territory as the index of territory quality.

Morphological Traits: For each adult we measured body mass and the length of four morphological characters: exposed culmen, beak from tip to nares, tarsus, and wing chord. We reduced these morphological measurements using principal components analysis into two orthogonal measures (accounting for 67% of the variation in these traits) signifying beak size (loadings: exposed culmen = 0.87; beak length to nares = 0.86; tarsus = 0.07; wing chord = -0.01) and body size (loadings: tarsus = 0.76; and wing chord = 0.78; exposed culmen = -0.01; beak length to nares = 0.08) after VARIMAX rotation (S4). Residual mass was calculated as the residual of mass regressed on the body size principal component.

Plumage Traits: Males molt from their cryptic winter plumage into dark breeding plumage during migration and have wing patches that are both larger and brighter than those of females. We measured five male plumage traits that vary considerably among individuals (Fig. 1) following methods described in (S4). Wing patch size was assessed as a rectangle by length and width (1999-2000) or as an area extracted from digital photographs (2001-2003) in Adobe Photoshop 5.5 (Adobe Systems Inc., San Jose CA). The two different measures were standardized (mean = 0, s.d. = 1) to allow comparison among years. Wing patch color was assessed from 2001-2003 by an Ocean Optics (Dunedin, FL) spectrometer with pulsed xenon light source, measuring wavelengths from 300-700nm. Raw data were converted to standard tristimulus variables (hue, chroma, and brightness) by ColoR software (S6). Our measure of wing patch color uses a principal component to combine values of overall brightness (loading = 0.85), UV chroma (loading = 0.74) and UV brightness (loading = 0.99) since all three measures are highly correlated (PCA accounted for 76% of the variation in these measures on a single axis). Color of black body feathers was scored into one of four rank categories of black coloration, on the basis of reference photographs, for four different body regions (head, nape, back, rump) and then combined into one measure of color with a principal components analysis (loadings: head =

0.63, nape = 0.73, back = 0.82, rump = 0.82; accounting for 66% of the variation in these traits on a single axis). These rank estimates of color were correlated with spectrometer measures of black coloration taken for the same individuals from 2001-2003 (Pearson's $r = 0.6$, $n = 337$, $P < 0.001$; *S4*). Finally, the proportion of black feathers versus brown feathers (i.e. patch size of black feathers) was assessed for two regions: the rump and the rest of the body excluding wings and tail (i.e., head, nape, back, and belly combined). The rump and the rest of the body were examined separately because they are distinct morphologically and our behavioral observations indicate that they are used in different behavioral contexts. All measures were significantly repeatable within a year for the same individuals measured multiple times (*S7*; repeatability = 0.63 - 0.92, $n = 34-65$, *S4*).

Social Dominance: Frequent behavioral observations provided data on the association between male traits and measures of social dominance (*S4*). First, we used the frequency of intrusions onto a territory holder's display area as an estimate of social dominance status prior to the arrival of females. This measure assumes that reduced intrusion rates reflect social dominance mediated through signaling traits (i.e. badges of status). Second, we compared the traits of the winners and losers of naturally occurring contests between two color-banded individuals. While these escalated contests may only occur in a subset of all birds, the comparison of traits should indicate a direct association between male traits and the ability to win a fight (i.e. social dominance). We also experimentally examined the link between the plumage characteristics of territory holders and their degree of social dominance by conducting experimental territorial intrusions using taxidermic models (1999-2002) to displaying territory holders (*S4*). Four different models were used to control for model effects, and there were no significant differences in response between models (*S4*). All presentations were conducted between 9am and 11am (sunrise was at roughly 6am) during the first two weeks of the field season (prior to female arrival). We scored the response of territorial males to this standardized intrusion using principal components analysis on response variables. The analysis yielded two VARIMAX rotated orthogonal axes with eigenvalue greater than 1 (*S4*). The first component reflects the speed and proximity of approach (loadings: time to first approach = -0.88, time to closest approach = -0.74, distance of first approach = -0.69, and distance of closest approach = -0.81; all other loadings < 0.1) to the model and the second reflects physical aggression (loadings: pecks = 0.70, kicks = 0.71, wing tilt = 0.74, bill tilt = 0.58, and bill wipe = 0.47; all other loadings < 0.2) towards the model. We then determined whether these two measures of a male's aggressive response to the model were correlated with his plumage characteristics (*S4*). Since these experiments used a standardized stimulus that did not change across years (i.e. the same taxidermic mounts), we also examined the responses of territorial males to the models to determine if the level of male-male aggression varied across years.

Paternity Analysis: To measure male fitness, including within pair and extra pair success, we conducted paternity analyses. We genotyped 529 progeny, 324 potential sires, and 154 nesting females using 6 hyper-variable microsatellite loci (Mme10, Mme12, Mme8, Mme2: *S8*, FhU2: *S9*, Gf06: *S10*). Fragments were amplified with standard PCR techniques with fluorescently labeled primers, analyzed on an ABI3100 sequencer with ROX500 size standard (Applied Biosystems, Foster City, CA), and all peaks were scored manually by ASC. We used Cervus software (*S11*) to exclude social mates as sires in each brood and assign paternity of extra pair offspring with 98% exclusion probability. Paternity was assigned at a conservative level ($P <$

0.05) on the basis of 100,000 run simulations that used actual allele frequency data, the capture rate of males (90% from field observations), and a 1% genotyping error rate (*S11*). We searched for extra-pair fathers in a sequential procedure, first including neighbors, and then more distant males where required (*S12*, *S13*). Significance tests by simulation require an estimate of the number of potential sires, but since neighborhood sizes varied for each male and for the level of analysis (neighbors vs. whole population), simulations were run separately for each year and each neighborhood size. With this technique, we were able to assign a sire to 86% of all nestlings (455/529) including 62% of all extra-pair nestlings at the $P < 0.05$ confidence level.

Fitness and Selection Analyses: We estimated the strength and form of selection on male traits using multiple regression with standardized traits (mean = 0, s.d. = 1) (*S14*, *S15*). Due to large sample sizes necessary to analyze models that included 7 or 8 male traits, we searched for the best model with the fewest number of factors to balance an accurate description of selection with recovered degrees of freedom in the error term. We performed best subsets Generalized Linear Models (GLMs) with Akaike's Information Criterion (AIC) to search for the best explanatory model in STATISTICA software (StatSoft Inc., USA). Models with the lowest AIC included only variables whose partial correlation coefficient was $P < 0.05$ (denoted on figures). However, our analyses yielded equally good models (*S16*, *S17*) containing other factors which suggests that additional traits may also have been the target of weak selection. We therefore present consensus models (*S16*, *S17*), including factors in all models with $\Delta AIC \leq 1$ in our figures and used these as final models in linear model estimation of selection coefficients to account for correlational effects on traits (*S14*). In all cases, model significance and selection coefficients were negligibly affected by inclusion of these factors relative to the 'best model'. Each GLM examining the relationship between male traits and a fitness estimate was fitted with the distribution that best fit the dependent variable combined with the appropriate link function: 1) choice of a social mate (mated / not mated) fitted with binomial errors and a logit link, 2) male fitness (total number of chicks a male sired that successfully fledged from both his own nests and the nests of other males) fitted with a Poisson distribution and logit link, and 3) female fitness (whether or not the nest fledged any young) with a binomial distribution and logit link (number of chicks fledged gives similar results using a Poisson distribution and logit link).

Randomization tests: We used randomization tests in two contexts: to search for overlap between the male traits under total sexual selection and those under selection due to pairing success across years, and to search for associations between traits selected for through female choice of a social mate and the male traits that predict female fitness (fledging success) across years. We asked if the number of significant selection coefficients (partial $P < 0.05$, and sharing the same sign) shared between female mate choice and male or female fitness in the same years was higher than random expectation. This method preserves the temporal component of variation in selection when looking for a match between mate choice and each form of fitness. Our method drew at random traits associated with the two different variables (choice and fitness) from all available traits and examined the degree of overlap (shared traits) between these two distributions. Years were treated as independent since selection patterns varied from year to year, so the maximum number of independent trait-years was 76 (7 traits for 5 years plus wing patch color in 3 years; this total is doubled to account for the sign, negative or positive, of each coefficient). The number of traits drawn in the randomization test was the same as the observed number of traits under selection when the results from the individual year analyses were all

summed (10 traits under significant selection for female choice based on Fig. 4; 12 traits under significant selection for male fitness from Fig 2; 7 traits under significant selection for female fitness from Fig S2). Null expectations were determined by selecting significant traits at random for both female choice and either male or female fitness for each year, tallying the total number of instances where the same trait was selected for in both mate choice and female fitness (correcting for the coefficient sign; 50% positive based on observed data), and adding up the matches for all years. We repeated this procedure 10,000 times and calculated the P value as the proportion of the 10,000 runs that had at least as many matches between female choice and either male or female fitness as the observed data (male fitness: 5 overlaps between Fig 2 and Fig 4; female fitness: 4 overlaps between Fig. 4 and Fig. S2). This test corresponds to a one-tailed test, which is appropriate since the expectation was to find only a high degree of similarity, not dissimilarity.

Cubic Splines: For splines describing variation in selection across years, we compiled data on male traits and pairing success or male total fitness separately for each year. To avoid ties in the data, we included the number of males with the same mating status and trait as a weighting factor (*S18*). To estimate the effects of selection over a longer time scale, data for all five years were combined, ties in male traits were resolved within years, and year was included as a covariate (exclusion of year had negligible effects). We used GLMS software (*S18*) to plot cubic splines of selection on male traits. We first searched for the value of λ (smoothing parameter) that minimized the generalized cross-correlation value (GCV), choosing the most conservative value (i.e. most linear) in cases of more than one minimum. Next, we ran GLMS with the best λ including 1000 bootstrap replicates to generate standard errors and assess fit of the splines. For most traits associated with pairing success, when selection occurred within a year it was linear (GCV score minimized at $\lambda > 9$; 35/38 possible quadratic effects in GLMs had $P > 0.1$) and interactions between traits were also rare (147/158 possible 2-way interactions in GLM have $P > 0.1$) so we focused on linear effects. Significance of splines was determined through GLM analyses described above and presented in Fig. 2 and Fig. 4.

SOM Text: Supporting Results

Male Social Dominance: Trait correlations with pairing success can reflect either direct female choice of a male with specific traits or female choice of resources (e.g. habitat quality) that are correlated with male traits that are used to establish male ownership over the resources that attract females. We have previously shown that some male traits are associated with measures of social dominance (*S4*). With such male-male competition, patterns of mate acquisition could be dynamic across years either because the habitat features females choose vary across years or because different male traits are associated with defending those resources in different years. We took two approaches to examine if male-male competition could drive variable pairing patterns. First, we examined the relationship between pairing patterns and habitat quality (availability of nest vegetation since most foraging occurs off site and territories themselves are not defended after mate acquisition). Habitat quality was never associated with pairing patterns, either in yearly selection analyses or analyses with data from all years combined (i.e. never occurred in final GLM models after AIC model selection corresponding to a partial $P > 0.2$). Second, we examined whether the traits used as aggressive signals (*S4*) differ across years in a manner that could account for the variable pairing patterns we found. Observational data indicate

that the male traits associated with reduced intrusion rates by other males did not differ across years (data from 1999-2003; GLM: year×male trait interactions, partial $P > 0.1$, $df = 4,184$ for all 7 traits studied). In addition male traits associated with winning pair-wise contests among males did not differ among years (data from 1999-2003; GLM: year×male trait interactions $P > 0.1$, $df = 4,41$ for all 7 traits; winner and loser matched by interaction). Using experimental intrusions with taxidermic models (described above) we looked for associations between the traits of a territory holder and his aggressive response towards an experimental intruder. Neither the association between a male's traits and his aggressive response nor his intensity of approach to the model differed among years (Data from 1999-2002; GLM: year×aggression $P > 0.1$, $df = 3,26$; year×approach intensity $P > 0.1$, $df = 3,29$). This suggests that in a standardized context, the traits that predict the intensity of a male's response to intrusion did not differ across years. Furthermore, the overall degree of response by male territory holders to standardized experimental intrusion using taxidermic models did not differ across years (Data from 1999-2002; ANOVA: $N = 45$, approach intensity $P = 0.7$ for latency, and aggression $P = 0.9$) suggesting that the intensity of male-male aggression and competition was similar across years. Taken together, these patterns suggest that male-male competition cannot explain the observed temporal variation in the male traits associated with social mating patterns. While male-male competition may play a role in the mating systems of this species, the observed temporal changes in mating patterns appear to largely reflect changes in direct female choice for male traits rather than resources males compete for.

Changes in Female Preferences: To determine the mechanism that caused the observed population level shifts in female preference across years, we evaluated alternative patterns that could account for this variability including 1) changes in the makeup of the female population due to immigration and emigration, 2) strong age-dependent shifts in female preference coupled with cohort effects, and 3) plasticity in the preferences of individual females (results of the latter reported in the main text).

Two patterns are required for large shifts in the makeup of the female population to drive population shifts in female preference: there must be high turnover of females (i.e. movement or death) and new females must have different preferences from the previous population. We cannot evaluate the first criterion since it is unclear what levels of turnover would be required to generate a population level pattern. However, we can reject this mechanism because the second criterion did not hold: the mate choice preferences of new females entering the population (i.e. unmarked females) did not differ from those of individually marked returning females (GLM: all $df = 1,125$, residual mass: $F = 7.59$, $P = 0.007$, mean \pm SE: new = -0.78 ± 0.22 , old = 0.86 ± 0.55 ; all other traits and year \times age $P > 0.1$).

In some species, female preferences change with age or experience (S19, S20). If such age-effects were coupled with a strong cohort effect (most females are the same age), population change in preference over time could result. This hypothesis was rejected by a lack of age-dependent (i.e. directional) change in trait preference for females that returned across years (repeated measures ANOVA all $df = 1,23$, all $P > 0.1$; Fig. S4), although returning females did prefer males larger than their previous mate (rmANOVA for body size $F_{1,23} = 7.41$, $P = 0.012$).

Supplemental Figures

Figure S1: Changes in the trait preferences of individual females that were observed with different mates across years varies considerably (24 across-year comparisons). Plotted are the absolute values of change in preferences for standardized male traits (units are s.d.). Tukey box plots show the median (center line), quartiles (box edges), and the range of values (whiskers or stars).

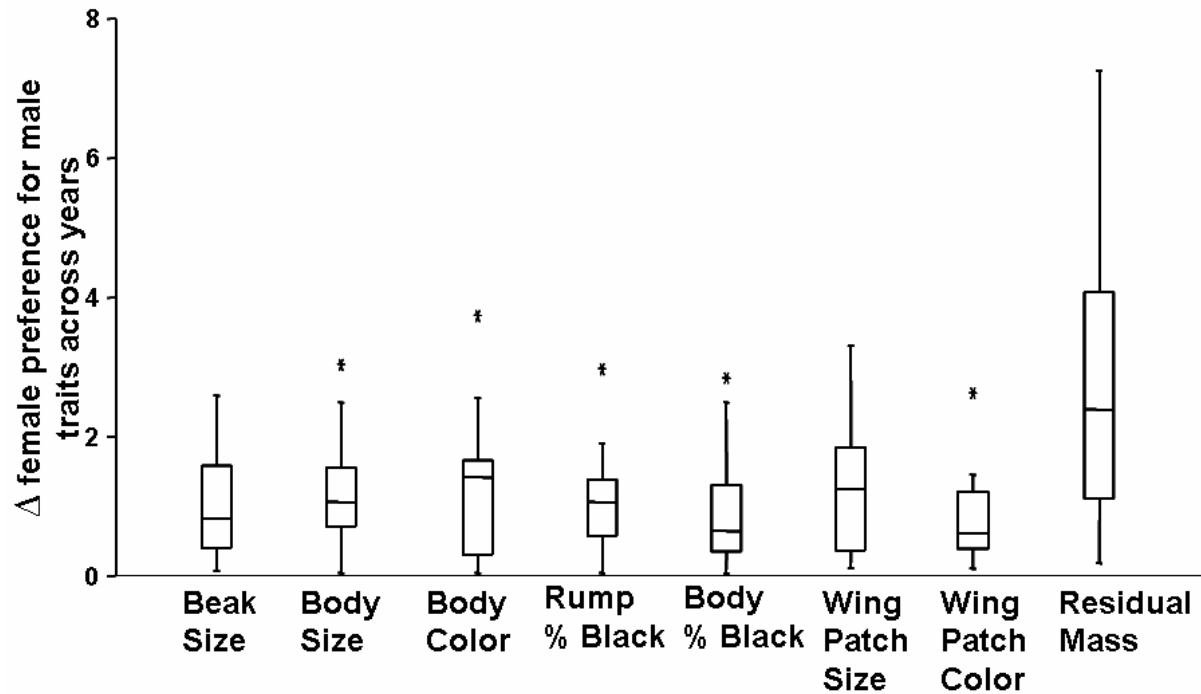


Figure S2: Association between male traits and success of the male's nest (fledged young/ did not fledge young), a measure of female reproductive success. Below the diagram for each year is the P -value and sample size from the final GLM model (see methods). Thickness of arrows between traits and nest success indicate the value of the partial correlation coefficient (selection gradient). Dashed and solid arrows are negative and positive relationships respectively. Traits include three morphometric measures (beak size, body size, and residual mass) and five plumage measures: body feather color (rank color), percent black feathers on the rump (rump%) and rest of the body (body%), wing patch size (WP size), and wing patch color (WP color). Factors that had a significant partial effect in GLMs designated as $*P < 0.05$, $+P < 0.1$.

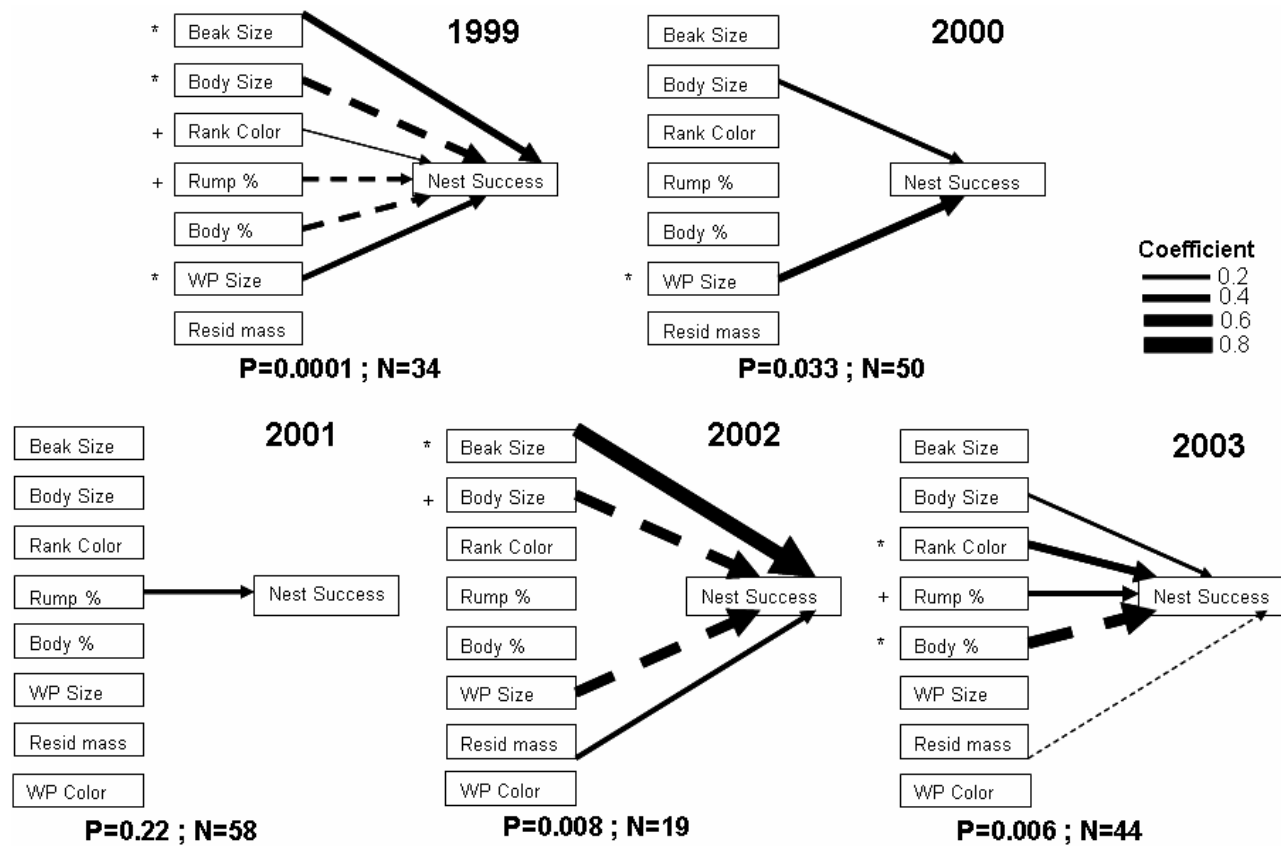


Figure S3: Cubic splines reveal the dynamic nature of selection on male traits due to changes across years in female choice of a social mate. Colored splines represent selection on male traits in a given year due to female mate choice while black splines represent selection in all years combined. Rank color and wing patch size show marked reversals in the direction of selection. Solid lines depict traits that entered into final selection models (P-values from Fig. 4; see methods) for a given year (or all years combined) whereas dashed lines depict traits that were not significant. Only residual mass and beak size show significant selection for analyses using data from all years combined.

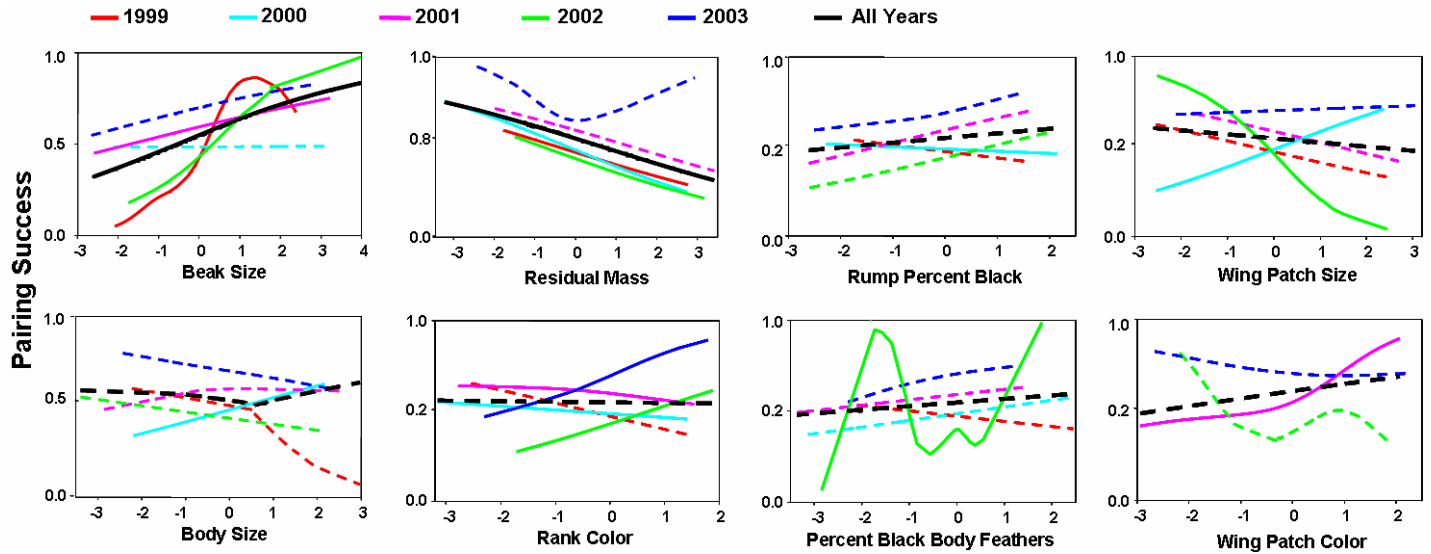
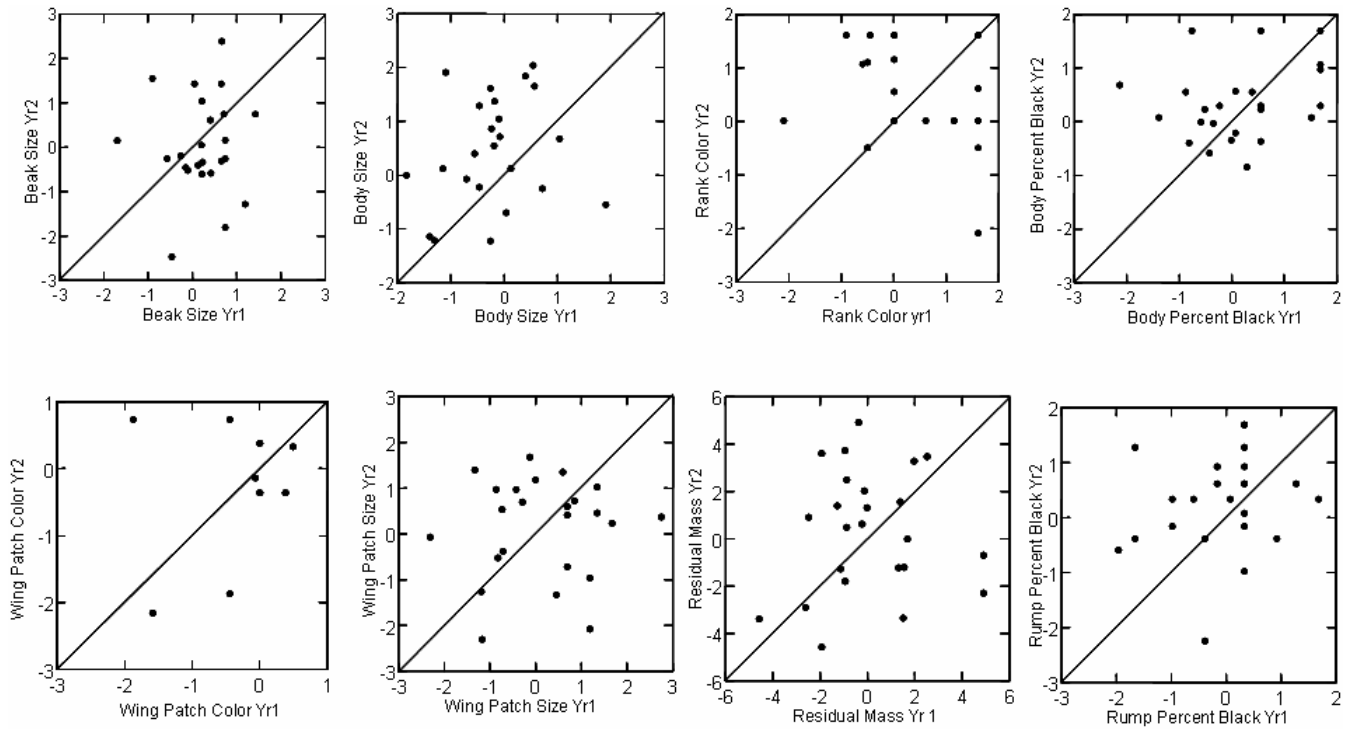


Figure S4: Lack of age-related changes in the traits of the mates of females that returned to the study site in more than one year. Significant age related patterns would have points concentrated either above or below the 1:1 line rather than equal scatter on each side. Only body size shows a significant age effect (see supplemental results above).



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