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Scale-of-choice effect in the assortative mating by multiple ornamental and non-ornamental characters in the black-headed gull

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Abstract
Assortative mating is a tendency to mate with phenotypically similar individuals as a consequence of either selection acting on mate preferences or individual constraints resulting from temporal and spatial segregation. The aim of this study was to examine the patterns of assortative mating in a plumage-monomorphic bird species, the black-headed gull *Chroicocephalus ridibundus*. For this purpose, we captured 217 pairs of gulls breeding in two colonies in northern Poland over six years and assessed whether they mated assortatively based on morphology, condition, individual genetic diversity, and expression of ornaments. An analysis of raw data (colonies and years combined) provided support for assortative mating by specific structural characters (head and wing morphology) and condition (size-corrected body mass). However, these relationships lost significance after removing inter-colony and inter-annual variation in the traits, suggesting that they were primarily driven by spatial and temporal segregation of individuals. We also found support for assortative mating by the size, but not coloration, of two melanin-based plumage ornaments (hood and wingtip). These correlations were retained after removing variation between colonies and years, suggesting that they could be due to directional mate preferences. No evidence was found for assortative mating by physiological measures of condition (blood hemoglobin concentration and plasma metabolite concentrations) and heterozygosity of neutrally selected microsatellite loci. Our study provides rare evidence for the scale-of-choice effect in assortative mating, where different variants of a given trait are distributed non-randomly across spatial or temporal scales and pooling them into a single heterogeneous sample may produce false-positive results.

Significance statement
Animals often choose their reproductive partners non-randomly, preferring to mate with individuals that are similar by certain phenotypic characters, which results in an assortative pattern of mating. However, similar patterns can occur as a result of non-random distribution of individuals across spatial and temporal scales. Here, we examined the patterns of assortative mating in a colonial waterbird, the black-headed gull *Chroicocephalus ridibundus*. We found that assortative mating by morphological characters and condition was apparent only in a heterogeneous sample, where data from different colonies and years were pooled together. In contrast, assortative mating by the size of melanin-based plumage ornaments (hood and wingtip) was retained after removing inter-colony and inter-annual variation in these traits. Our study provides a rare example of the scale-of-choice effect in assortative mating of birds.

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Introduction

Animals can either mate randomly or they may show a tendency to mate with phenotypically similar or dissimilar

individuals, which is recognized as assortative and disassortative mating, respectively. While disassortative mate choice is generally rare within the animal kingdom, assortative mating has been reported for several major invertebrate and vertebrate taxonomic groups (Jiang et al. 2013). Assortative mating has been hypothesized to evolve as a result of selection acting on mate preferences either directly or indirectly via its effect on offspring fitness (Kirkpatrick 2000; Albert and Schluter 2004). On the other side, an alternative hypothesis assumes that assortative mating may not be favored by selection, but it can arise as a mere consequence of individual constraints resulting from temporal and spatial segregation or intra-sexual competition (Sutherland et al. 2007; Snowberg and Bolnick 2012). Whatever is the mechanism responsible for the evolution of assortative mating, evolutionary consequences of this phenomenon are far from negligible. At the population level, assortative mating increases homozygosity and produces linkage disequilibrium between loci, which may inflate the variance of quantitative traits (Jiang et al. 2013). On a larger evolutionary scale, assortative mating facilitates sympatric speciation, especially in combination with disruptive selection that eliminates the intermediate genotypes (Kondrashov and Mina 1986; Doebeli 1996). However, under certain conditions, a stringent invariant assortative mating may be sufficient to complete sympatric speciation in the absence of natural selection, as well as it may able to prevent the merging of species after their secondary contact (Kondrashov and Shpak 1998).

A recent meta-analysis spanning five phyla of animals showed that assortative mating in birds is relatively weak when compared to some other taxonomic groups, such as fish or crustaceans (Jiang et al. 2013). Though, assortative mating in birds has been found to occur by a wide spectrum of phenotypic traits including an overall size (Urfi and Kalam 2006), specific structural characters (Merilä and Sheldon 2000), body condition (Potti 2007), age (Cézilly et al. 1997; Brown and Roth 2009), ornament quality (Jawor et al. 2003; MacDougall and Montgomerie 2003), and behavior (Filliater and Breitwisch 1997). However, studies on mate choice in birds usually examined the patterns of assortative mating by a set of similar, usually either structural or visual, traits (reviewed in Jiang et al. 2013). In contrast, information on assortative mating by a combination of multiple different phenotypic characters (such as size, condition, ornaments, or age) is generally lacking for most avian species (Jiang et al. 2013). Also, phenotypic and genetic characters have rarely been combined in the research on mate assortment in wild bird populations (García-Navas et al. 2009). Inter-specific data suggest that different trait categories show large heterogeneity in the strength of assortative mating, which may reflect different mechanisms responsible for the occurrence of mating assortment by each trait (Jiang et al. 2013). Similar variation is also expected within-species, as some traits are subject to strong

sexual selection (assortative mating resulting from directional mate preferences), while the others are not (assortative mating resulting from temporal or spatial segregation). Thus, focusing on a single trait or a set of similar traits from the same category is, by far, insufficient to gain a complete picture of mating patterns within any population.

The aim of this study was to investigate assortative mating by multiple phenotypic and genetic characters in a colonial waterbird, the black-headed gull *Chroicocephalus ridibundus* (Laridae, Charadriiformes). Specifically, we tested whether black-headed gulls mated assortatively by the following traits:

(a) body size and structural characters not related directly to size, including head and wing morphology;
(b) body condition, as measured with size-corrected body mass, blood hemoglobin concentration, and plasma concentrations of basic metabolites;
(c) size and spectral properties of three putative plumage ornaments: hood, wingtip, and eye ring;
(d) genetic heterozygosity measured across a set of microsatellite markers.

We also examined whether assortative mating patterns in our black-headed gull population were driven by the scale-of-choice effect (SCE). The concept of SCE assumes that different variants of a given trait are non-randomly distributed across spatial or temporal scales and pooling them into a single heterogeneous sample may produce false-positive results on assortative mating (Rolán-Alvarez et al. 2015). To assess the SCE, we compared the strength of assortative mating calculated for raw data pooled across two breeding colonies and six years to the strength of assortative mating after removing inter-colony and inter-annual variation in each trait.

Material and methods

General field procedures and morphological measurements

Fieldwork was conducted between 2011 and 2016 in two breeding colonies of the black-headed gull in northern Poland. The first colony was located on a lake island at the outskirts of a medium-sized city, Bydgoszcz (53°10′ N, 18°02′ E; 176 km^2, 360,000 inhabitants). The lake was relatively small (10.2 ha), and birds were observed to primarily forage at nearby meadows and in the Brda river valley (4–5 km away from the colony). The second colony was located in an agricultural landscape north of Bydgoszcz, where gulls nested on a lake near Kusowo village (53°15′ N, 18°02′ E). Birds were observed feeding either on the breeding lake, two other nearby lakes, or in the surrounding farmland. The colonies held 1100–1450 and 850–1100 pairs, for Bydgoszcz and

Kusowo, respectively. Gulls were captured during incubation on nests using spring traps (Ecotone, Sopot, Poland). In total, we captured 217 pairs of black-headed gulls (Bydgoszcz: $N = 43$ pairs; Kusowo: $N = 174$ pairs; Table S1 in Electronic Supplementary Material). All individuals were captured between 20 April and 11 June, and trapping procedures caused no losses in broods. Although birds were not individually banded, each year, we captured only a small percentage of pairs from the colonies (1–2% in Bydgoszcz and 2–9% in Kusowo), and each year, pairs were randomly sampled throughout the entire colonies (central and peripheral zones) and across different nesting densities. We also avoided catching birds at the same nests in successive years, so likelihood of capturing the same individuals in different years was relatively low. Thus, pseudoreplication should not pose a problem for our analyses of traits that were measured across multiple years in each colony (morphological characters and size-corrected body mass). Random sampling was also applied to obtain subsamples for the analyses of physiological condition, ornamental characters, and heterozygosity. In general, black-headed gulls show high rate of natal dispersal (e.g. 38% across several French colonies; Péron et al. 2010), so sampling of unknown kin-related birds should not constitute a major bias in our analyses, especially when considering a large number of colonies and a large overall population size of black-headed gulls in our study area (over 100,000 breeding pairs in Poland; Tomiałojć and Stawarczyk 2003). Although year-to-year mate retention in the black-headed gull is possible due to nest-site fidelity, pair bonds are not maintained throughout a year and pair formation occurs in the colonies just prior to breeding (Cramp and Simmons 1983). Thus, within-pair correlations at the initial stage of breeding, as examined in our study, are expected to reflect actual mate choice rather than constitute a byproduct of shared environment.

We collected a set of basic morphological measurements from each captured individual. Tarsus length (TL) and total head length (THL) were measured with calipers (± 0.01 mm), while wing length (WL) was measured with a stopped ruler (± 0.5 mm). We also measured vane lengths of the second (P2) and the third (P3) primary with a standard ruler (± 0.5 mm). Each bird was weighed with a spring balance (± 2 g). All these measurements were collected for 212–217 pairs, depending on the trait measured (Table S1 in the Electronic Supplementary Material, ESM). Additionally, we used calipers to measure head width (HW; ± 0.01 mm) in 40 pairs (Table S1 in the ESM). To reduce variability, all individuals were measured by one of the authors (PI). In order to calculate a composite measure of body size of captured individuals, we reduced three basic morphological measurements (THL, TL, and WL) to the first principal component of the principal component analysis (PCA). All three measurements showed moderate positive correlations with each other ($0.47 < r < 0.53$; all $P < 0.001$). PC1 accounted for 66.1% of

the variability in the three morphological characters, and all univariate measurements had similar contributions to PC1 (0.32 to 0.34). PC1 showed strong positive correlations with each of the univariate measurement ($0.79 < r < 0.82$; all $P < 0.001$).

Since sex of black-headed gulls cannot be identified by plumage, we collected about 20 μl of blood from the ulnar vein of each bird to FTA ClassicCards (Whatman, Maidstone, UK) for the purpose of molecular sexing. The same samples were used for microsatellite analyses. FTA cards provide an effective matrix to preserve blood in order to process the isolation of genomic DNA and can be stored for several years at room temperature without any precautions (Gutiérrez-Corchero et al. 2002). Thus, all collected blood sampled put on the cards were dried and stored at room temperature until analysis. In 2016, we collected additional blood samples from birds breeding in the Kusowo colony for the purpose of physiological and biochemical analyses of blood parameters ($N = 87$ pairs). Approximately 5 μl of blood was collected for hemoglobin measurements, and 20–40 μl of blood was taken into heparinized capillary tubes for plasma metabolite measurements. All capillary tubes were kept on ice for up to 6 h and then centrifuged at 6000 rpm for 5 min. The plasma was separated and kept at −20 °C until analyzed. Although it was not possible to apply blinded methods for the collection of morphological measurements and blood/feather samples in the field, all further laboratory analyses (molecular sex determination, microsatellite genotyping, blood plasma analyses, and measurements of ornamental characters) were performed by individuals uninformed about the research question.

Condition estimates

We estimated body condition using three different methods. First, we adjusted body mass for structural size using the scaled mass index, which was calculated with the formula developed by Peig and Green (2009):

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{\text{SMA}}}$$

where M_i and L_i are the body mass and the linear body measurement of individual i, respectively; b_{SMA} is the scaling exponent estimated by the standardized major axis regression of body mass on linear body measurement; L_0 is the mean value of the linear body measurement for the study population; and $\hat{M}_i$ is the predicted body mass for individual i when the linear body measure is standardized to L_0. Among the three basic morphological measurements (THL, TL, and WL), THL showed the strongest log-log correlation with body mass in both males ($r = 0.33$, $N = 212$, $P < 0.001$) and females ($r = 0.34$, $N = 212$, $P < 0.001$). This indicated that THL best explained fraction of mass associated with structural size (Peig

and Green 2009) and, thus, it was used to calculate the scaled mass index. The mean value of THL (L_0) was 82.09 ± 0.20 mm. b_{SMA} = 1.992 ± 0.077 (R^2 = 0.38) was estimated with the RMA software (Bohonak 2004) using 100,000 bootstraps.

Second, we assessed whole-blood hemoglobin concentration, which is a general measure of blood oxygen-carrying capacity in vertebrates (Minias 2015). Although in mammals the potential of an organism to satisfy its oxygen demand is more appropriately measured with the amount of hemoglobin per unit surface area of erythrocytes (Kostelecka-Myrcha 2002), in birds, the changes of total surface area of erythrocytes proceed proportionally to the changes of hemoglobin concentration (Kostelecka-Myrcha 1997). Hence, whole-blood hemoglobin concentration reliably indicates capacity of an avian organism to satisfy its oxygen needs and, as such, it has been reported to correlate with a broad spectrum of fitness-related traits in birds, including survival, condition, and developmental stability (reviewed in Minias 2015). Also, experimental studies on passerines showed that hemoglobin concentration increased with diet quality (Pryke et al. 2011; Pryke and Rollins 2012). Thus, hemoglobin concentration was recommended as a robust indicator of physiological condition in birds (Minias 2015). Hemoglobin concentrations were measured using a portable HemoCue Hb 201+ photometer (HemoCue, Ängeholm, Sweden). For this purpose, about 5 µl of blood of each bird was collected into a disposable HemoCue microcuvette, where it mixed with reagents. The absorbance, directly proportional to hemoglobin concentration, was measured within 10 min from blood sampling.

Third, we measured plasma concentrations of five basic metabolites: total protein, albumin, triglycerides, glucose, and uric acid. Plasma chemistry is considered a valuable tool to assess physiological condition in wild-living birds (Jenni-Eiermann and Jenni 1998). Plasma concentrations of basic metabolites have been reported to correlate with food intake, diet quality, refueling rate during migration, fat reserves, and size-adjusted body mass in a large spectrum of avian species (e.g. Totzke et al. 1999; Lyons et al. 2008). All plasma metabolite concentrations were analyzed with BTS-330 spectrophotometer (BioSystems Reagents & Instruments, Barcelona, Spain) using commercial kits of the same manufacturer (BioSystems Reagents & Instruments, Barcelona, Spain). All analyses were conducted according to the manufacturer's protocols using the following methods: total protein (biuret reaction), albumin (bromocresol green), triglycerides (glycerol phosphate oxidase/peroxidase), glucose (glucose oxidase/peroxidase), and uric acid (uricase/peroxidase). Absorbance of each sample was measured in a flow cuvette against a blank reagent. Since the quantity of collected plasma was not always sufficient to measure concentrations of all five metabolites, the final sample sizes for each parameter differed between 85 and 74 pairs of gulls (Table S1 in the ESM).

Ornamental characters

We examined three putative ornaments in the black-headed gull: hood, wingtip, and eye ring. Based on human perception, black-headed gulls are sexually monomorphic in plumage, which is dominated by white and gray coloration. Brownish hood and black wingtip (Fig. 1) are the only contrasting melanin-based plumage parts in the breeding plumage of black-headed gulls (Baker 1993). In general, there is accumulating empirical evidence that melanin-based ornaments can act as an honest signal of individual quality (Roulin 2016) and hence be subject to sexual selection and play a role in mate selection (Jawor and Breitwisch 2003). Furthermore, brownish hood of the black-headed gulls is developed only for the reproductive season and the timing of its appearance in spring well correlated with the timing of laying and clutch size of females (van Rhijn and Groothuis 1987). Thus, we hypothesized that the size and coloration of brown hood are particularly likely to constitute honest ornamental traits in the black-headed gull. Furthermore, large number of studies suggests that the size of achromatic white plumage patches may signal individual quality or condition and be under sexual selection (e.g. Pärt and Qvarnström 1997; Hanssen et al. 2006). For this reason, we also measured the size of the structurally white eye ring, which may act as an additional ornament, beacuse it shows considerable inter-individual variation (PI, person. observ.) and is well exposed on the dark background of the hood.

We measured the size of three putative ornamental characters (lateral area of the hood, black tip of the second primary, and white eye ring) in 43 pairs of black-headed gulls captured in 2013–2014 in both colonies (Bydgoszcz: N = 23; Kusowo: N = 20). We used a digital camera (Canon EOS 400D with 18–55 mm lens) to take photographs of the lateral view of the head and the second primary from a distance of 30 cm (Fig. 1). A millimeter paper (grid 1 mm^2) was used as a background for all the photographs. AutoCAD 2014 software (AutoDesk, San Rafael, CA, USA) was used to outline contours and calculate the size of all putative ornaments. Due to insufficient quality of some photographs, the final sample sizes varied between 29 and 38 measured gull pairs per each ornamental trait (Table S1 in the ESM). Sizes of hood and eye ring were independent of the total head length in both sexes (Pearson's correlations; all P > 0.15), while the size of melanized area at the second primary was independent of the second primary length (P > 0.6).

Reflectance spectrophotometry

Reflectance was measured for two melanin-based putative plumage ornaments, i.e. brown hood and black tip of the second primary. We also measured reflectance for white neck feathers, as coloration of white achromatic plumage has been reported to signal quality in some avian species (Doucet et al.

Fig. 1 Digital photographs of three putative ornamental characters in the black-headed gull: brownish hood (**a**), white eye ring (**a**), and black tip of the second primary (**b**). Contours for area measurements of each ornament are shown with white (**a**) and yellow (**b**) solid lines
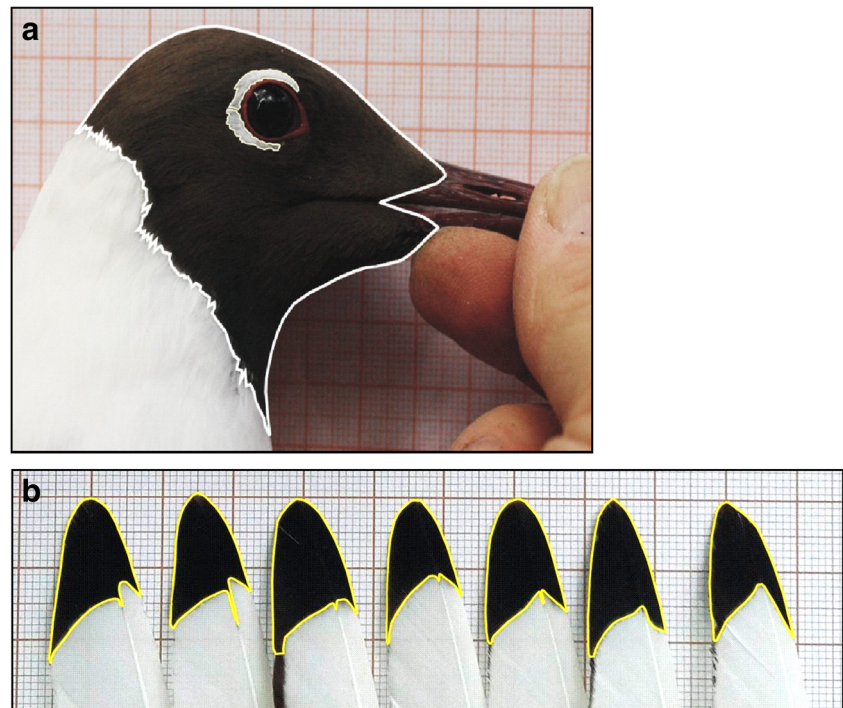
2005; Guindre-Parker et al. 2012). For the purpose of the measurements, we plucked the left second primary ($N = 36$ pairs), as well as ca. 15 hood and neck feathers, respectively ($N = 37$ pairs). Prior to measurements, hood and neck feathers were taped to black matte paper and arranged in a manner that mimics their arrangement on the birds' skin (Quesada and Senar 2006). Primaries were laid on black velvet before the measurements. Spectral reflectance of feathers (300–700 nm) was measured using a set of USB4000 spectrometer and deuterium-halogen light source (DH-2000-BAL) connected with a fiber-optic measuring probe (R 200-7-UV/VIS; Ocean Optics, Dunedin, FL, USA). The probe was held at a 90° angle to the feather's surface at the fixed distance of 1.5 mm and, thus, illuminated an area of approximately 2 mm in diameter. Before measuring each sample, measurements were standardized using a white standard (WS-1-SL, Labsphere, North Sutton, NH, USA), while the dark standard was taken by turning off the light source and covering the probe. We took three readings per each ornament per individual. Consecutive measurements were at least 2 mm apart from each other. All measurements were performed by one of the authors (KK).

We processed spectral data using the RCLR v0.9.28 software (Montgomerie 2008). For hood and wingtip, we calculated two reflectance variables, brightness and red chroma, which are commonly used in studies on melanin-based coloration (Siefferman and Hill 2005; Surmacki et al. 2015). Brightness was calculated as the mean reflectance for each wavelength (1 nm) between 300 and 700 nm (B2 in RCLR software; Montgomerie 2008), which is considered a good predictor of

the total amount of melanin deposited in brownish or black feathers (McGraw et al. 2005). Red chroma was calculated as the proportion of reflectance in the red range of the spectrum (600–700 nm) to the total reflectance at 300–700 nm (S1R in RCLR software; Montgomerie 2008), and it was used to characterize a relative contribution of pheomelanin to an overall melanin-based coloration (McGraw et al. 2005; Zduniak et al. 2014). Structurally white coloration of neck feathers was assessed with brightness (B2) and UV chroma (S1U in the RCLR software; Montgomerie 2008), which expresses the proportion of UV reflectance (300–400 nm) in the total reflectance. Brightness and UV chroma are known to accurately describe variance of structurally white coloration (e.g. Doucet et al. 2005). All color variables were separately calculated for three reflectance measurements per trait per individual. Within-individual repeatability was assessed using intra-class coefficients that were calculated with the *irr* package (Gamer et al. 2012) in R statistical environment (R Development Core Team 2013). Although repeatability values were low to moderate (Table S2 in the ESM), they were highly significant (all $P < 0.001$), and, thus, repeated measurements of each color variable were averaged within each ornament of each individual. Averaged reflectance curves are presented in Fig. S1 (ESM).

DNA extraction and molecular sexing

Nuclear DNA was extracted from the blood stored on the FTA cards using Bio-Trace DNA Purification Kit (EURx, Gdańsk, Poland). For this purpose, a piece of dried blood sample

(approx. 2 mm^2) was cut away from each card with a sterile cutter. After extraction, amplifications of the chromo-helicase-DNA-binding (CHD) region were performed with the primer pair 2550F and 2718R (Fridolfsson and Ellegren 1999) according to the protocols described by Griffiths et al. (1998). Annealing temperature was set to 50 °C. Products were separated on 2% agarose gels. A male was indicated by one band (approx. 650 bp), and a female produced two bands (approx. 650 and 450 bp).

Microsatellite genotyping and heterozygosity estimates

Estimates of heterozygosity in the black-headed gull were based on six microsatellite loci (RBG13, RBG18, RBG20, RBG27, RBG28, RBG29) originally developed for red-billed gull *Larus novaehollandiae scopulinus* (Given et al. 2002). All these loci were previously found to successfully cross-amplify and to show moderate polymorphism in the black-headed gull (Ležalová-Piálková 2011). PCR amplifications were conducted in a final volume of 20 μl containing 10 μl of DreamTaq PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 μl of DNA template, and 0.2 μM of each primer. Annealing temperatures and PCR conditions followed the protocol developed by Given et al. (2002). Fragment size analysis was conducted with ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored against the GeneScan™ 600 LIZ Standard (Applied Biosystems) using the Geneious 10.0.5 software (Biomatters Ltd., Auckland, New Zealand).

Microsatellite genotyping was conducted for 53 pairs of black-headed gull captured in 2016 in Kusowo. Each locus showed between 7 and 26 alleles, and the observed heterozygosity ranged from 0.43 to 0.93 (Table 1). We used ARLEQUIN 1.3.5.2 software (Excoffier and Lischer 2010) to test for agreement of allele frequencies with Hardy-Weinberg equilibrium (exact tests by Guo and Thompson 1992) and for linkage disequilibrium between all pairs of loci (10,000 permutations). After Bonferroni's correction for multiple comparisons, we found no evidence for departure of any locus from Hardy-Weinberg equilibrium or for significant linkage disequilibrium between any pairs of loci. No evidence for null alleles and scoring errors due to stuttering or large allele dropout was found with the MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004).

We used four measures of heterozygosity in microsatellite markers. First, multi-locus heterozygosity (MLH) was calculated as the number of heterozygous loci divided by the total number of loci. Second, d^2 was calculated as a mean squared difference in allele sizes within each locus (Coulson et al. 1998), which has been reported to capture distant inbreeding (Forstmeier et al. 2012). Third, we calculated homozygosity

Table 1 Characterization of six polymorphic microsatellite loci in the black-headed gull breeding in northern Poland ($N = 106$ individuals). N_A, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity

Locus	N_A	Size range (bp)	H_e	H_o
RBG13	9	207–225	0.66	0.62
RBG18	7	172–184	0.42	0.43
RBG20	20	172–218	0.90	0.89
RBG27	26	186–246	0.91	0.93
RBG28	8	160–176	0.74	0.78
RBG29	8	127–141	0.78	0.82

by locus as HL = $(\Sigma E_h)/(\Sigma E_h + \Sigma E_j)$, where E_h and E_j are the expected heterozygosities of the loci that an individual bear in homozygosis (h) and in heterozygosis (j), respectively (Aparicio et al. 2006). Thus, HL weighs the contribution of each locus to individual homozygosity based on the allelic variability (Aparicio et al. 2006). Finally, internal relatedness (IR) was calculated as a modified relatedness coefficient of Queller and Goodnight (1989) using the following formula: IR = $(2H - \Sigma f_i) / (2 N - \Sigma f_i)$, where H is the number of homozygous loci, N is the number of loci, and f_i is the frequency of the ith allele contained in the genotype (Amos et al. 2001). Since HL and IR measure homozygosity, we reversed their signs prior to analyses, so that higher values indicated higher heterozygosity.

Statistical analyses

The analysis of assortative mating in the black-headed gull was conducted in three major steps. First, relationships in all measured traits between mated males and females were examined using Pearson's product-moment correlation coefficients calculated for raw data (colonies and years combined). Second, we removed inter-colony variation in all the traits that were measured across the two colonies (morphological measurements, ornamental characters, and the scaled mass index; Table S1 in the ESM) by extracting residuals from the analysis of variance with the effect of colony included as a fixed factor. Residuals were extracted separately for males and females. Pearson product-moment correlation coefficients were calculated for these residuals to examine the strength of assortative mating while controlling for the spatial segregation of individuals among the colonies. For traits that remained under positive assortative mating after removing inter-colony variation, we examined whether the strength of assortative mating varied between the two studied colonies. For this purpose, we calculated Pearson's product-moment correlation coefficients separately for the two colonies and compared them using z tests. Third, we simultaneously removed inter-colony and inter-annual variation in all traits measured in multiple years in

the two colonies (scaled mass index and all morphological traits except for the head width; Table S1 in the ESM) by extracting residuals from the analysis of variance with the effects of colony and year entered as fixed factors. Since data from each colony were collected in different years, the effect of year was nested within the colony. Again, we calculated the Pearson's product-moment correlation coefficients for these residuals to examine the strength of assortative mating while controlling for the spatial (inter-colony) and temporal (inter-annual) segregation of individuals. Because predictions of our four main hypotheses (assortative mating by morphology, condition, ornaments, and heterozygosity) would be satisfied by a single trait per hypothesis, we adjusted all the P values for the false discovery rate in the multiple testing separately for each trait category using the approach developed by Benjamini and Hochberg (1995). Distributions of all measured traits, except for plasma triglyceride concentration, were reasonably close to normal (absolute skewness and kurtosis < 2). The distribution of plasma triglyceride concentrations was strongly right-skewed (skewness 2.06), and, thus, it was log-transformed prior to analyses. t tests were used to assess inter-colony differences in morphological measurements, ornamental characters, and the scaled-mass index. Paired t tests were used to test for differences in all measured traits between mated males and females. All statistical analyses were performed with the STATISTICA 10.0 (StatSoft, Tulsa, OK, USA).

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information.

Results

Males were significantly larger than their female partners in all morphological measurements (Table S2 in the ESM). Between-sex differences in condition were found for SMI and plasma triglyceride concentration, both of which were significantly higher in females than males (Table S3 in the ESM). No significant intersexual variation was found for other condition indices (Table S3 in the ESM). Hood area was significantly larger in males than in female partners, while the other ornamental characters showed no significant intersexual differences (Table S3 in the ESM). No differences in heterozygosity were found between sexes (Table S3 in the ESM).

An analysis of raw data pooled across colonies and years provided no evidence for assortative mating by a composite measure of body size (PC1) in the black-headed gull (Table 2), although individuals mated assortatively by several specific morphological traits. The strongest assortative mating was found for the head width, total head length, and P3 length

($r = 0.37$, $r = 0.33$, and $r = 0.32$, respectively; Table 2; Fig. 2). We also found significant assortative mating by wing length and P2 length, but no evidence for assortative mating by tarsus length was found (Table 2). However, after removing inter-colony variation, significant assortative mating was found only for P3 length ($r = 0.26$; Table 2; Fig. 2) while all the other relationships lost significance (Table 2), suggesting that they were attributed to non-random distribution of individuals among the colonies. In fact, birds nesting in Kusowo had significantly longer wings and shorter heads than birds from Bydgoszcz (Table S4 in the ESM). Comparison of correlation coefficients calculated separately for Kusowo and Bydgoszcz colonies indicated that the strength of assortative mating by P3 length did not differ significantly between the two sites ($P = 0.08$). Nevertheless, after removing inter-colony and inter-annual variation at the same time, we found no significant correlation for P3 length (Table 2; Fig. 2), indicating that this relationship was driven by annual variation in primary lengths.

While analyzing raw data, we found that black-headed gulls also mated assortatively by condition (Table 2). Specifically, there was a significant positive correlation between the scaled mass index of mated pairs of black-headed gulls ($r = 0.25$; Table 2; Fig. 2). However, we found significant inter-colony differences in the scaled mass index of both males and females (Table S4 in the ESM), indicating that birds from the Kusowo colony were in better condition. After removing this variation, intra-pair correlation for the scaled mass index lost significance (Table 2; Fig. 2), suggesting that it was driven by spatial segregation of birds among the colonies. An analysis of raw data provided no evidence for assortative mating by whole-blood hemoglobin concentration or plasma metabolite concentrations (Table 2).

We found support for assortative mating by two putative ornamental characters in the black-headed gull. An analysis of raw data provided support for a strong positive correlation between hood area of mated pairs of gulls ($r = 0.56$; Table 2; Fig. 3) and a moderately strong correlation between the size of P2 melanized tip ($r = 0.40$; Table 2; Fig. 3). Both relationships remained significant after removing inter-colony variation (Table 2; Fig. 3), and the strength of assortative mating was similar for the two colonies ($P = 0.80$ and $P = 0.53$ for hood and P2 tip, respectively). We found no evidence for assortative mating by the size of eye ring and coloration of the hood, P2 tip, and neck (Table 2). No evidence was found for assortative mating by any of heterozygosity estimates (Table 2).

Discussion

An analysis of assortative mating across colonies and years provided support for positive intra-pair correlations for specific structural characters (head and wing morphology) and

Table 2 Correlations between morphological measurements, ornamental characters, condition estimates, and heterozygosity of mated pairs of black-headed gulls. Sample sizes (N), Pearson's product-moment correlation coefficients (r), and unadjusted P values (P) are shown. Asterisks indicate statistical significance after controlling for the false discovery rate (FDR) in multiple testing ([NS] $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Significant results (after controlling for FDR) are marked in bold

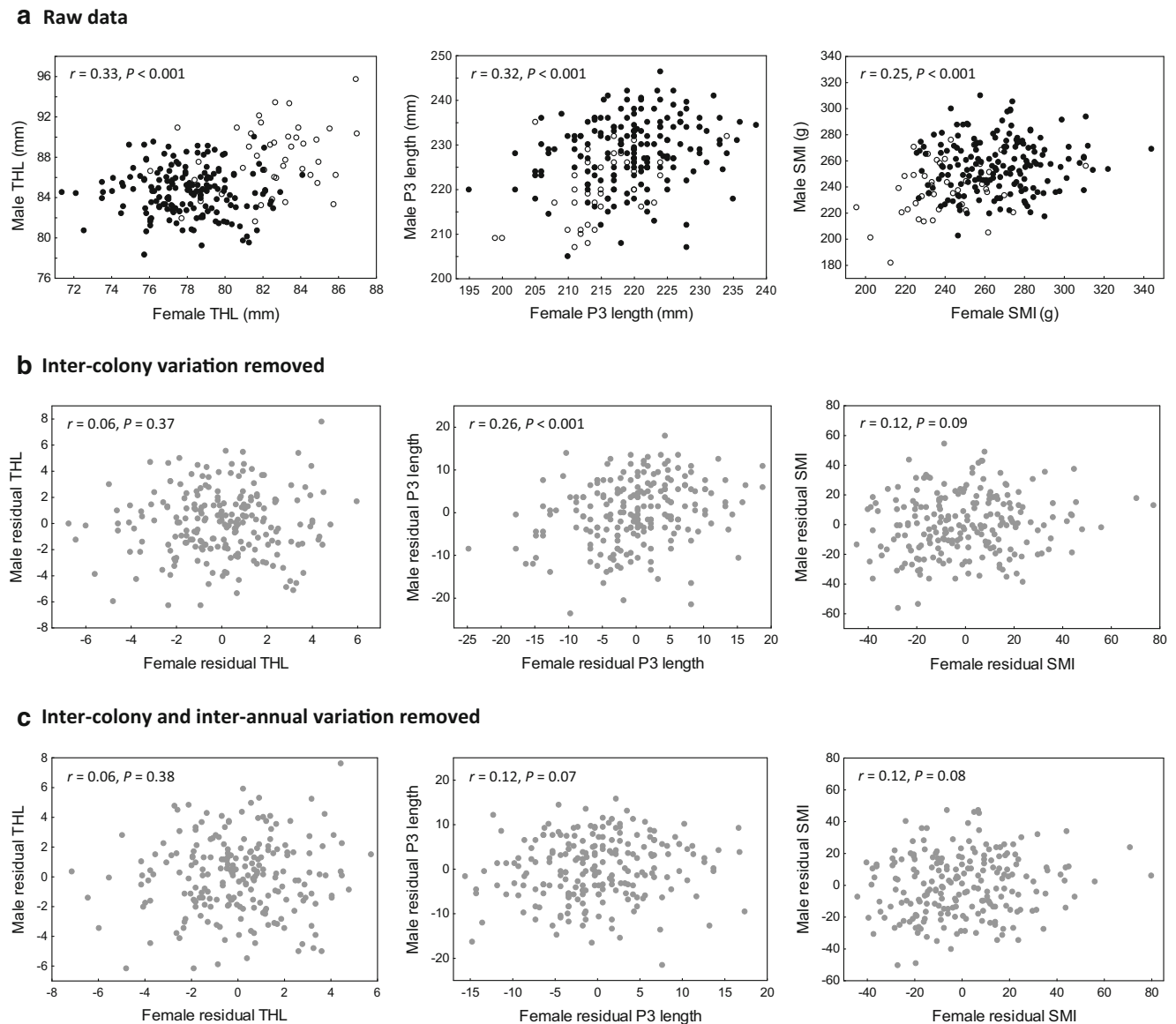| Trait	N	Raw data		Inter-colony variation removed		Inter-colony and inter-annual variation removed	
		r	P	r	P	r	P
Morphological characters							
Overall size (PC1)	211	− 0.02	0.72	− 0.04	0.54	− 0.08	0.24
Wing length	214	**0.16**	**0.016***	0.10	0.16	0.08	0.26
Tarsus length	217	0.06	0.41	0.05	0.45	− 0.03	0.69
Total head length	214	**0.33**	**< 0.001***	0.06	0.37	0.06	0.38
Head width	40	**0.37**	**0.018***	0.38	0.017[NS]	–	–
P2 length	216	**0.16**	**0.020***	0.13	0.052	0.10	0.14
P3 length	216	**0.32**	**< 0.001***	**0.26**	**< 0.001***	0.12	0.072
Ornamental characters							
Hood area	38	**0.56**	**< 0.001**	**0.48**	**0.002***	–	–
P2 melanized area	39	**0.40**	**0.011***	**0.41**	**0.010***	–	–
Eye ring area	30	0.04	0.83	0.00	0.98	–	–
Hood brightness	37	0.06	0.74	− 0.10	0.56	–	–
Hood red chroma	37	− 0.19	0.26	− 0.19	0.26	–	–
P2 brightness	37	0.04	0.81	− 0.16	0.36	–	–
P2 red chroma	37	0.12	0.48	0.19	0.27	–	–
Neck brightness	37	− 0.19	0.25	− 0.19	0.26	–	–
Neck UV chroma	37	− 0.14	0.42	− 0.13	0.43	–	–
Condition estimates							
Scaled mass index	212	**0.25**	**< 0.001***	0.12	0.087	0.12	0.075
Hemoglobin	87	0.17	0.11	–	–	–	–
Total plasma protein	85	− 0.02	0.87	–	–	–	–
Plasma albumin	85	0.05	0.64	–	–	–	–
Plasma triglycerides	80	− 0.03	0.85	–	–	–	–
Plasma glucose	78	0.03	0.79	–	–	–	–
Plasma uric acid	74	− 0.10	0.40	–	–	–	–
Heterozygosity estimates							
MLH	53	− 0.19	0.17	–	–	–	–
d^2	53	0.23	0.10	–	–	–	–
IR	53	− 0.14	0.32	–	–	–	–
HL	53	− 0.18	0.18	–	–	–	–

condition (the scaled mass index). However, all these relationships disappeared when we removed inter-colony and inter-annual variation in the traits, suggesting that they were primarily driven by non-random distribution of different trait variants across space and time, thus, being consistent with the scale-of-choice effect. In contrast, positive intra-pair correlations for the size melanin-based plumage ornaments (hood and wingtip) were retained after controlling for spatio-temporal variation in their distribution.

So far, the most extensive data on assortative mating in birds have been collected for morphological characters (reviewed in Jiang et al. 2013). Consistent with our results for the black-headed gull, relatively few studies on birds reported assortative mating by composite measures of body size. As far as we are aware, evidence for positive assortative

mating by structural size within the order of Charadriiformes was found only for the parasitic skua *Stercorarius parasiticus*, which displays reversed sexual size dimorphism (Catry et al. 1999). This suggests that gulls and allies may not choose their mates based on an overall body size and that large body size may be of limited fitness advantage, at least in one of the sexes. In contrast, assortative mating by head and bill morphology (total head length, culmen length, bill length, and bill depth) was found in several species of larids from *Chlidonias* and *Sterna* genera (Coulter 1986; Stern and Jarvis 1991; Ledwoń 2011), as well as in other chradriiform clades, such as auks (Wagner 1999) or scolopacids (Hedenström 1987). In our study, we found positive intra-pair correlations for head morphology (total head length and head width) while pooling raw data from different colonies and years. However, these

a Raw data

b Inter-colony variation removed

## c Inter-colony and inter-annual variation removed

Fig. 2 Total head length (THL), length of the third primary (P3 length), and the scaled mass index (SMI) of mated pairs of black-headed gulls, as shown with raw data (**a**), residuals against the effect of colony (**b**), and residuals against the effects of colony and year (**c**). Raw data are shown with white and black points for Bydgoszcz and Kusowo colony, respectively, while residuals are shown with gray points. Significant correlations indicate positive assortative mating

relationships were exclusively attributed to non-random distribution of individuals among the colonies, as both males and females had significantly larger heads in Bydgoszcz than in Kusowo. This pattern could reflect habitat-related differences in the size or type of prey available at the two sites, as birds from Bydgoszcz foraged primarily on meadows, while birds from Kusowo searched for prey on lakes and in the farmland. In gulls and terns, head size determines the effectiveness of capturing large prey by increasing the snapping power of the bill (Ashmole 1968; Ledwoń 2011), so possibly better availability of larger prey in Bydgoszcz could favor settlement of bird with larger heads. However, we lack any qualitative data

on prey type and abundance at the two sites to provide any empirical support for this hypothesis.

Similar mechanism could be responsible for assortative mating by the size-corrected body mass, which was only apparent while pooling data across colonies and years. There were marked differences in the scaled mass index between Bydgoszcz and Kusowo colonies, and after removing this variation we found no significant assortative mating. We also found no evidence for assortative mating by several measures of physiological condition. In general, assortative mating by condition has rarely been documented in birds. Positive correlations between size-corrected body mass of mated pairs

a Raw data
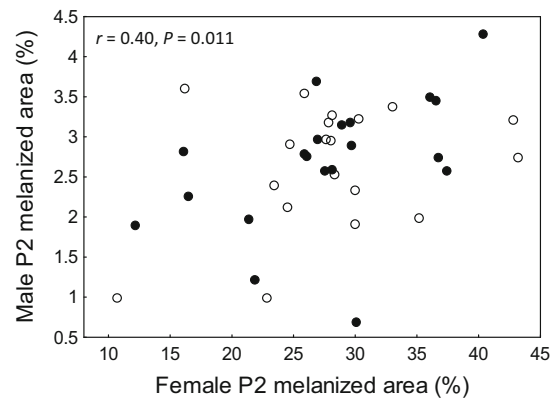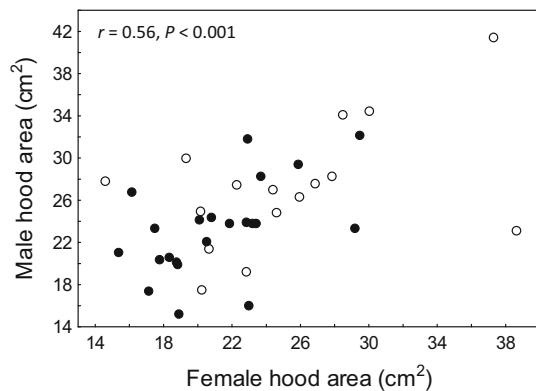
b Inter-colony variation removed
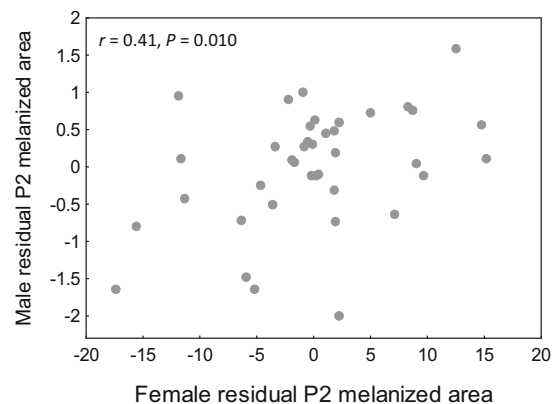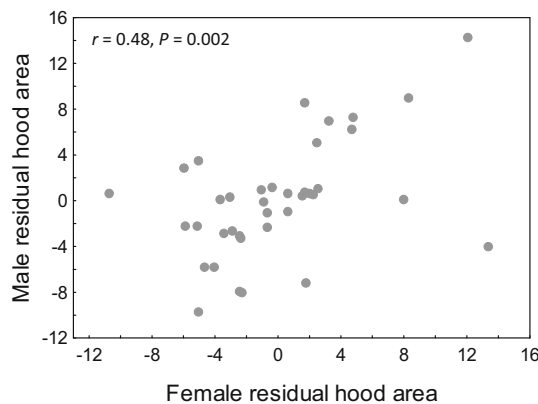
Fig. 3 Melanized area of two putative plumage ornaments, hood and the tip of the second primary (P2), of mated pairs of black-headed gull, as shown with raw data (**a**) and residuals against the effect of colony (**b**).

Raw data are shown with white and black points for Bydgoszcz and Kusowo colony, respectively, while residuals are shown with gray points. Significant correlations indicate positive assortative mating

have been reported for a handful of species (Masello and Quillfeldt 2003; Tryjanowski and Šimek 2005; Silva et al. 2008). Even less evidence exists for assortative mating by physiological measures of condition in birds. An extensive review of assortative mating by Jiang et al. (2013) reported only two such cases, where pied flycatchers *Ficedula hypoleuca* mated assortatively by hematocrit (Potti 2007) and Adélie penguins *Pygoscelis adeliae* mated assortatively by their oxidative status (Beaulieu et al. 2010).

An analysis of pooled data also showed significant intra-pair correlations for wing morphology, as measured with wing length, as well as the lengths of P2 and P3 primaries. While the correlations for wing length and P2 length were explained by non-random distribution of individuals among the colonies, assortative mating by P3 length was still apparent after removing inter-colony variation in this trait. Nevertheless, we found that this relationship was driven by significant inter-annual variation in average primary lengths within the colonies. Black-headed gulls molt primaries once a year. Primary molt is commenced on nesting grounds (May–June) and may be continued throughout the post-breeding period, usually till

October (Baker 1993). During this period, adult birds may still reside in breeding colonies or in their close vicinity and, thus, they are likely to experience similar environmental conditions during feather growth. There is large body evidence that avail-ability and quality of food during molt can affect the length, mass, and structure of growing feathers (Murphy et al. 1988; Waite 1990; Pap et al. 2008). Thus, during the poor years (in terms of feeding conditions), birds are likely to grow slightly shorter and lighter feathers than during the good years. This can produce significant inter-annual variation in average pri-mary lengths within colonies, which would rather be unex-pected for skeletal measurements that often show little intra-individual variation from year to year (Jensen et al. 2003). Also, primary lengths can be under different selective pres-sures in different years, which could possibly contribute to the inter-annual variation in this trait. Primary lengths are indica-tive of wing shape, which determines flight performance (Swaddle and Lockwood 2003) and migration strategies of individuals (Saino et al. 2010; Minias et al. 2013). As adaptive value of different migration strategies can vary with environ-mental conditions in a given year, we can expect that birds

with various wing shapes would show differential survival in different years. However, other studies of assortative mating in larids failed to reveal positive intra-pair correlations in wing morphology, despite pooling data from multiple years (Helfenstein et al. 2004; Ledwoń 2011), suggesting lack of clear between-year variation in the distribution of wing length or shape.

In contrast to morphological characters and condition, assortative mating by the size of two putative melanin-based plumage ornaments, the hood and the tip of the second primary, was not explained by spatial (between-colony) and temporal (between-year) segregation of birds. Although assortative mating by size of ornaments was likely to arise via directional mate preferences, our correlational approach could not exclude within-year temporal segregation or fine-scale (microhabitat) spatial segregation of these traits in the studied black-headed gull colonies. Hood and wingtip are the only contrasting (strongly melanized) parts within the breeding plumage of black-headed gulls, which is otherwise dominated by white and light gray coloration. We found no support for assortative mating by brightness and red chroma of these ornaments, nor by the size of white eye ring. Similarly, no assortative mating was found for brightness and UV chroma of the white neck feathers. Although there is abundant evidence for assortative mating by ornamental characters in birds, most research focused on carotenoid-based (Jawor et al. 2003; MacDougall and Montgomerie 2003) or structural (Komdeur et al. 2005) coloration of plumage and non-plumage ornaments. Assortative mating by the size of a melanin-based ornament was previously found in the European shag *Phalacrocorax aristotelis*, where mates were paired assortatively with respect to black crest size (Daunt et al. 2003). Assortative mating by melanin-based coloration (brightness and UV chroma) has been reported for the collared flycatcher *Ficedula albicollis* (Laczi et al. 2011). Whereas in both these cases, the pattern of assortative mating by melanin-based ornaments was unaffected by age-related variation in the expression of the ornaments, we cannot exclude that assortative mating by age could drive, at least to some extent, positive correlations between ornament size of mated pairs in our population of black-headed gulls. It is widely acknowledged that the expression of ornaments may increase with age in birds (e.g. Budden and Dickinson 2009; Freeman-Gallant et al. 2010), and assortative mating by age has been recorded in a wide range of avian species (reviewed in Jiang et al. 2013). Thus, it is often difficult to separate the effects of the relative influences of age and ornamentation on mate selection (Komdeur et al. 2005). Since we lack information on the age of black-headed gulls from our population, we cannot reject the hypothesis that age effects mediated assortative mating by ornamental characters in our studied population. However, there is also good evidence from the literature that mutual ornaments (such as the hood and wingtip in the black-

headed gull) can have a signal function in both sexes and directional mate preferences in males and females (both sexes prefer to pair with a highly ornamented partners) can result in strong assortative mating within populations (Kraaijeveld et al. 2007). Interestingly, contrary to the size of melanin-based ornaments, their spectral properties were not a subject of assortative mating. This pattern could be due to low between-individual variation of melanin ornaments reflectance, which is supported by modest repeatability of spectrometric variables (Table S2 in the ESM). It is, thus, possible that in the case of dark plumage traits like hood or primary tip in the black-headed gull, the signal is more efficiently conveyed by their size than coloration.

Finally, we found no support for assortative mating by heterozygosity of black-headed gulls, as measured across a set of microsatellite loci (so-called heterozygous mate hypothesis; Brown 1997). Heterozygosity is generally linked to individual quality, so choosing a more heterozygous partner may provide direct benefits in the form of increased territory size, fecundity, or parental care (reviewed in Kempenaers 2007). Also, heterozygosity is expected to be correlated between parents and offspring (Hoffman et al. 2007), so mates of heterozygous individuals may gain indirect, heritable fitness benefits. There is accumulating evidence that heterozygosity may be reflected by ornaments (e.g. Ferrer et al. 2015), which provides a reliable tool to assess individual genetic diversity of potential mates. However, empirical evidence for assortative mating by heterozygosity across neutrally selected loci is scarce in birds (García-Navas et al. 2009; Ortego et al. 2009). The possible explanation for this fact is that heterozygosity at neutral loci correlates with fitness only in inbred populations, where it reflects genome-wide heterozygosity, which in turn correlates with individual inbreeding coefficient (Slate et al. 2000). Even then, most heterozygosity-fitness correlations are weak, unless variation in individual inbreeding coefficients within populations is high (Slate et al. 2004) or populations are strongly substructured (Balloux et al. 2004). The European population of the black-headed gull is estimated at 1.5–2 mln breeding pairs (BirdLife International 2017), and no evidence exists for recent genetic bottlenecks in this species. This may suggest lack of heterozygosity-fitness correlations and, consequently, lack of selection towards mate choice by heterozygosity in the black-headed gulls.

In conclusion, our results provided a rare example of the scale-of-choice effect in assortative mating of birds, where positive intra-pair correlations for morphological characters and size-corrected body mass were due to non-random distribution of different variants of a given trait across spatial or temporal scales. Also, our study provided one of the first evidence for positive assortative mating by melanin-based plumage ornaments (hood and wingtip). Intra-pair correlations for the size of the ornaments were not explained by the spatial (inter-colony) and temporal (inter-annual) segregation of

individuals, suggesting that they could be the result of directional mate preferences. Our study highlights an importance of sample homogeneity across space and time in the studies of assortative mating in wild-living animals.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was performed under the permissions of the Local Bioethical Commission for Animal Welfare at the UTP University of Science and Technology in Bydgoszcz (31/2014), the Regional Environmental Protection Directorate in Bydgoszcz (WPN.6401.1.96. 2014.MP), and the General Environmental Protection Directorate in Poland (DOP-OZGIZ.6401.03.135.2012.km2 and DOP-OZGIZ.6401. 03.135.2012.km2). All experiments complied with the current laws of Poland (Act on Nature Conservation from 16 April 2004, Journal of Laws from 2004, No. 92, item 880).

References

Albert AYK, Schluter D (2004) Reproductive character displacement of male stickleback mate preferences: reinforcement or direct selection? Evolution 58(5):1099–1107. https://doi.org/10.1111/j.0014-3820.2004.tb00443.x

Amos W, Wilmer JW, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. Proc R Soc Lond B 268(1480):2021–2027. https://doi.org/10.1098/rspb.2001.1751

Aparicio JM, Ortego J, Cordero PJ (2006) What should we weigh to estimate heterozygosity, alleles or loci? Mol Ecol 15(14):4659–4665. https://doi.org/10.1111/j.1365-294X.2006.03111.x

Ashmole NP (1968) Body size, prey size and ecological segregation in five sympatric tropical terns (Aves: Laridae). Syst Zool 17(3):292–304. https://doi.org/10.2307/2412008

Baker K (1993) Identification guide to European non-passerines. British Trust for Ornithology, Thetford

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? Mol Ecol 13:3021–3031

Beaulieu M, Ropert-Coudert Y, Le Maho Y, Ancel A, Criscuolo F (2010) Foraging in an oxidative environment: relationship between δ^{13}C values and oxidative status in Adélie penguins. Proc R Soc Lond B 277(1684):1087–1092. https://doi.org/10.1098/rspb.2009.1881

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

BirdLife International (2017) Species factsheet *Larus ridibundus*, http://www.birdlife.org

Bohonak AJ (2004) RMA: software for reduced major axis regression, http://www.bio.sdsu.edu/pub/andy/RMA.html

Brown JL (1997) A theory of mate choice based on heterozygosity. Behav Ecol 8(1):60–65. https://doi.org/10.1093/beheco/8.1.60

Brown WP, Roth RR (2009) Age-specific reproduction and survival of individually marked wood thrushes, *Hylocichla mustelina*. Ecology 90(1):218–229. https://doi.org/10.1890/07-2061.1

Budden AE, Dickinson JL (2009) Signals of quality and age: the information content of multiple plumage ornaments in male western bluebirds *Sialia mexicana*. J Avian Biol 40(1):18–27. https://doi.org/10.1111/j.1600-048X.2008.04344.x

Catry P, Phillips RA, Furness RW (1999) Evolution of reversed sexual size dimorphism in skuas and jaegers. Auk 116(1):158–168. https://doi.org/10.2307/4089462

Cézilly F, Boy V, Tourenq CJ, Johnson AR (1997) Age-assortative pairing in the greater flamingo *Phoenicopterus ruber roseus*. Ibis 139:331–336

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH (1998) Microsatellites reveal heterosis in red deer. Proc R Soc Lond B 265(1395):489–495. https://doi.org/10.1098/rspb.1998.0321

Coulter MC (1986) Assortative mating and sexual dimorphism in the common tern. Wilson Bull 98:93–100

Cramp S, Simmons KEL (1983) The birds of the western palearctic, vol vol. 3. Oxford University Press, Oxford

Daunt F, Monaghan P, Wanless S, Harris MP (2003) Sexual ornament size and breeding performance in female and male European shags *Phalacrocorax aristotelis*. Ibis 145:54–60

Doebeli M (1996) A quantitative genetic competition model for sympatric speciation. J Evol Biol 9(6):893–909. https://doi.org/10.1046/j.1420-9101.1996.9060893.x

Doucet SM, Mennill DJ, Montgomerie R, Boag PT, Ratcliffe LM (2005) Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. Behav Ecol 16(1):218–222. https://doi.org/10.1093/beheco/arh154

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10(3):564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Ferrer ES, García-Navas V, Bueno-Enciso J, Sanz JJ, Ortego J (2015) Multiple sexual ornaments signal heterozygosity in male blue tits. Biol J Linn Soc 115(2):362–375. https://doi.org/10.1111/bij.12513

Filliater TS, Breitwisch R (1997) Nestling provisioning by the extremely dichromatic northern cardinal. Wilson Bull 109:145–153

Forstmeier W, Schielzeth M, Mueller JC, Ellegren H, Kempenaers B (2012) Heterozygosity-fitness correlations in zebra finches: microsatellite markers can be better than their reputation. Mol Ecol 21(13):3237–3249. https://doi.org/10.1111/j.1365-294X.2012.05593.x

Freeman-Gallant CR, Taff CC, Morin DF, Dunn PO, Whittingham LA, Tsang SM (2010) Sexual selection, multiple male ornaments, and age- and condition-dependent signaling in the common yellowthroat. Evolution 64(4):1007–1017. https://doi.org/10.1111/j.1558-5646.2009.00873.x

Fridolfsson AK, Ellegren H (1999) A simple and universal method for molecular sexing of nonratite birds. J Avian Biol 30(1):116–121. https://doi.org/10.2307/3677252

Gamer M, Lemon J, Fellows I, Singh P (2012) irr: various coefficients for interrater reliability and agreement. R package ver. 0.84, https://CRAN.R-project.org/package=irr

García-Navas V, Ortego J, Sanz JJ (2009) Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. Proc R Soc Lond B 276(1669):2931–2940. https://doi.org/10.1098/rspb.2009.0417

Given AD, Mills JA, Baker AJ (2002) Isolation of polymorphic microsatellite loci from the red-billed gull (*Larus novaehollandiae scopulinus*) and amplification in related species. Mol Ecol Notes 2(4):416–418. https://doi.org/10.1046/j.1471-8286.2002.00261.x

Griffiths R, Double MC, Orr K, Dawson RJ (1998) A DNA test to sex most birds. Mol Ecol 7(8):1071–1075. https://doi.org/10.1046/j.1365-294x.1998.00389.x

Guindre-Parker S, Gilchrist HG, Baldo S, Doucet SM, Love OP (2012) Multiple achromatic plumage ornaments signal multiple receivers. Behav Ecol 24:672–682

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48(2):361–372. https://doi.org/10.2307/2532296

Gutiérrez-Corchero F, Arruga MV, Sanz L, Garcia C, Hernandez MA, Campos F (2002) Using FTA cards to store avian blood samples for genetic studies. Their application in sex determination. Mol Ecol Notes 2:75–77

Hanssen SA, Folstad I, Erikstad KE (2006) White plumage reflects individual quality in female eiders. Anim Behav 71(2):337–343. https://doi.org/10.1016/j.anbehav.2005.04.021

Hedenström A (1987) Assortative mating in the little ringed plover Charadrius dubius. Ornis Scand 18(4):325–327. https://doi.org/10.2307/3676905

Helfenstein F, Danchin E, Wagner RH (2004) Assortative mating and sexual size dimorphism in black-legged kittiwakes. Waterbirds 27(3):350–354. https://doi.org/10.1675/1524-4695(2004)027[0350:AMASSD]2.0.CO;2

Hoffman JI, Forcada J, Trathan PN, Amos W (2007) Female fur seals show active choice for males that are heterozygous and unrelated. Nature 445(7130):912–914. https://doi.org/10.1038/nature05558

Jawor JM, Breitwisch R (2003) Melanin ornaments, honesty, and sexual selection. Auk 120(2):249–265. https://doi.org/10.1642/0004-8038(2003)120[0249:MOHASS]2.0.CO;2

Jawor JM, Linville SU, Beall SM, Breitwisch R (2003) Assortative mating by multiple ornaments in northern cardinals (Cardinalis cardinalis). Behav Ecol 14(4):515–520. https://doi.org/10.1093/beheco/arg041

Jenni-Eiermann S, Jenni L (1998) What can plasma metabolites tell us about the metabolism, physiological state and condition of individual birds? An overview. Biol Cons Fauna 102:312–319

Jensen H, Sæther B-E, Ringsby TH, Tufto J, Griffith SC, Ellegren H (2003) Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (Passer domesticus). J Evol Biol 16(6):1296–1307. https://doi.org/10.1046/j.1420-9101.2003.00614.x

Jiang Y, Bolnick DI, Kirkpatrick M (2013) Assortative mating in animals. Am Nat 81:E125–E138

Kempenaers B (2007) Mate choice and genetic quality: a review of the heterozygosity theory. Adv Stud Behav 37:189–178. https://doi.org/10.1016/S0065-3454(07)37005-8

Kirkpatrick M (2000) Reinforcement and divergence under assortative mating. Proc R Soc Lond B 267(1453):1649–1655. https://doi.org/10.1098/rspb.2000.1191

Komdeur J, Oorebeek M, van Overveld T, Cuthill IC (2005) Mutual ornamentation, age, and reproductive performance in the European starling. Behav Ecol 16(4):805–817. https://doi.org/10.1093/beheco/ari059

Kondrashov AS, Mina MV (1986) Sympatric speciation: when is it possible? Biol J Linn Soc 27(3):201–223. https://doi.org/10.1111/j.1095-8312.1986.tb01734.x

Kondrashov AS, Shpak M (1998) On the origin of the species by means of assortative mating. Proc R Soc Lond B 265(1412):2273–2278. https://doi.org/10.1098/rspb.1998.0570

Kostelecka-Myrcha A (1997) The ratio of amount of haemoglobin to total surface area of erythrocytes in birds in relation to body mass, age of nestlings and season of the year. Physiol Zool 70(3):278–282. https://doi.org/10.1086/639597

Kostelecka-Myrcha A (2002) The ratio of amount of haemoglobin to total surface area of erythrocytes in mammals. Acta Theriol 47(S1):209–220. https://doi.org/10.1007/BF03192488

Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J (2007) The evolution of mutual ornamentation. Anim Behav 74(4):657–677. https://doi.org/10.1016/j.anbehav.2006.12.027

Laczi M, Török J, Rosivall B, Hegyi G (2011) Integration of spectral reflectance across the plumage: implications for mating patterns. PLoS One 6(8):e23201. https://doi.org/10.1371/journal.pone.0023201

Ledwoń M (2011) Sexual size dimorphism, assortative mating and sex identification in the whiskered tern Chlidonias hybrida. Ardea 99(2):191–198. https://doi.org/10.5253/078.099.0209

Ležalová-Piálková R (2011) Molecular evidence for extra-pair paternity and intraspecific brood parasitism in the black-headed gull. J Ornithol 152(2):291–295. https://doi.org/10.1007/s10336-010-0581-1

Lyons JE, Collazo JA, Guglielmo CG (2008) Plasma metabolites and migration physiology of semipalmated sandpipers: refueling performance at five latitudes. Oecologia 155(3):417–427. https://doi.org/10.1007/s00442-007-0921-x

Masello JF, Quillfeldt P (2003) Body size, body condition and ornamental feathers of burrowing parrots: variation between years and sexes, assortative mating and influences on breeding success. Emu 103(2):149–161. https://doi.org/10.1071/MU02036

MacDougall AK, Montgomerie R (2003) Assortative mating by carotenoid-based plumage colour: a quality indicator in American goldfinches, Carduelis tristis. Naturwissenschaften 90(10):464–467. https://doi.org/10.1007/s00114-003-0459-7

McGraw KJ, Safran RJ, Wakamatsu K (2005) How feather colour reflects its melanin content. Funct Ecol 19(5):816–821. https://doi.org/10.1111/j.1365-2435.2005.01032.x

Merilä J, Sheldon B (2000) Lifetime reproductive success and heritability in nature. Am Nat 155(3):301–310. https://doi.org/10.1086/303330

Minias P (2015) The use of hemoglobin concentrations to assess physiological condition in birds: a review. Conserv Physiol 3:cov007

Minias P, Kaczmarek K, Włodarczyk R, Janiszewski T (2013) Wing shape influences stopover strategies in a migratory shorebird, the common snipe. Condor 115(3):535–542. https://doi.org/10.1525/cond.2013.120137

Montgomerie R (2008) RCLR, version 0.9.28. Queen's University, Kingston, Canada

Murphy ME, King JR, Lu J (1988) Malnutrition during the postnuptial molt of white-crowned sparrows: feather growth and quality. Can J Zool 66(6):1403–1413. https://doi.org/10.1139/z88-206

Ortego J, Calabuig J, Bonal R, Muñoz A, Aparicio JM, Cordero PJ (2009) Temporal variation of heterozygosity-based assortative mating and related benefits in a lesser kestrel population. J Evol Biol 22(12):2488–2495. https://doi.org/10.1111/j.1420-9101.2009.01865.x

Pap PL, Vágási CI, Czirják GÁ, Barta Z (2008) Diet quality affects postnuptial molting and feather quality of the house sparrow (Passer domesticus): interaction with humoral immune function. Can J Zool 86(8):834–842. https://doi.org/10.1139/Z08-060

Pärt T, Qvarnström A (1997) Badge size in collared flycatchers predicts outcome of male competition over territories. Anim Behav 54(4):893–899. https://doi.org/10.1006/anbe.1997.0514

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118(12):1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Péron G, Lebreton J-D, Crochet P-A (2010) Breeding dispersal in black-headed gull: the value of familiarity in a contrasted environment. J Anim Ecol 79(2):317–326. https://doi.org/10.1111/j.1365-2656.2009.01635.x

Potti J (2007) Variation in haematocrit of a passerine bird across life stages is mainly of environmental origin. J Avian Biol 38(6):726–730. https://doi.org/10.1111/j.2007.0908-8857.04073.x

Pryke SR, Rollins LA (2012) Mothers adjust offspring sex to match the quality of the rearing environment. Proc R Soc Lond B 279(1744): 4051–4057. https://doi.org/10.1098/rspb.2012.1351

Pryke SR, Rollins LA, Griffith SC (2011) Context-dependent sex allocation: constraints on the expression and evolution of maternal effects. Evolution 65(10):2792–2799. https://doi.org/10.1111/j.1558-5646.2011.01391.x

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43(2):258–275. https://doi.org/10.1111/j.1558-5646.1989.tb04226.x

Quesada J, Senar JC (2006) Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the great tit *Parus major*. J Avian Biol 6:609–616

R Development Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org

Rolán-Alvarez E, Carvajal-Rodríguez A, de Coo A, Cortés B, Estévez D, Ferreira M, González R, Briscoe AD (2015) The scale-of-choice effect and how estimates of assortative mating in the wild can be biased due to heterogeneous samples. Evolution 69(7):1845–1857. https://doi.org/10.1111/evo.12691

Roulin A (2016) Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. Biol Rev 91(2):328–348. https://doi.org/10.1111/brv.12171

Saino N, Rubolini D, von Hardenberg J, Ambrosini R, Provenzale A, Romano M, Spina F (2010) Spring migration decisions in relation to weather are predicted by wing morphology among trans-Mediterranean migratory birds. Funct Ecol 24(3):658–669. https://doi.org/10.1111/j.1365-2435.2009.01659.x

Siefferman L, Hill GE (2005) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). Evolution 59(8):1819–1828. https://doi.org/10.1111/j.0014-3820.2005.tb01828.x

Silva N, Avilés J, Danchin E, Parejo D (2008) Informative content of multiple plumage-coloured traits in female and male European rollers. Behav Ecol Sociobiol 62(12):1969–1979. https://doi.org/10.1007/s00265-008-0628-y

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE, McEwan JC (2004) Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. Heredity 93(3):255–265. https://doi.org/10.1038/sj.hdy.6800485

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH (2000) Inbreeding depression influences lifetime reproductive success in a wild population of red deer (*Cervus elaphus*). Proc R Soc Lond B 267(1453):1657–1662. https://doi.org/10.1098/rspb.2000.1192

Snowberg LK, Bolnick DI (2012) Partitioning the effects of spatial isolation, nest habitat, and individual diet in causing assortative mating within population of threespine stickleback. Evolution 66(11):3582–3594. https://doi.org/10.1111/j.1558-5646.2012.01701.x

Stern MA, Jarvis RL (1991) Sexual dimorphism and assortative mating in black terns. Wilson Bull 103:266–271

Surmacki A, Stepniewski J, Stepniewska M (2015) Juvenile sexual dimorphism, dichromatism and condition-dependent signaling in a bird species with early pair bonds. J Ornithol 156(1):65–73. https://doi.org/10.1007/s10336-014-1108-y

Sutherland DL, Hogg ID, Waas JR (2007) Is size assortative mating in *Paracalliope fluviatilis* (Crutacea: Amphipoda) explained by male-male competition or female choice. Biol J Linn Soc 92(1):173–181. https://doi.org/10.1111/j.1095-8312.2007.00839.x

Swaddle JP, Lockwood R (2003) Wingtip shape and flight performance in the European Starling *Sturnus vulgaris*. Ibis 145(3):457–464. https://doi.org/10.1046/j.1474-919X.2003.00189.x

Tomiałojć L, Stawarczyk T (2003) The avifauna of Poland. Distribution, numbers and trends. PTPP Pro Natura, Wrocław

Totzke U, Fenske M, Hüppop O, Raabe H, Schach N (1999) The influence of fasting on blood and plasma composition of herring gulls (*Larus argentatus*). Physiol Biochem Zool 72(4):426–437. https://doi.org/10.1086/316675

Tryjanowski P, Šimek J (2005) Sexual size dimorphism and positive assortative mating in red-backed shrike *Lanius collurio*: an adaptive value? J Ethol 23(2):161–165. https://doi.org/10.1007/s10164-004-0142-2

Urfi AJ, Kalam A (2006) Sexual size dimorphism and mating pattern in the pained stork (*Mycteria leucocephala*). Waterbirds 29(4):489–496. https://doi.org/10.1675/1524-4695(2006)29[489:SSDAMP]2.0.CO;2

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Resour 4(3):535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

van Rhijn J, Groothuis T (1987) On the mechanism of mate selection in black-headed gulls. Behaviour 100(1):134–169. https://doi.org/10.1163/156853987X00116

Wagner RH (1999) Sexual size dimorphism and assortative mating in razorbills (*Alca torda*). Auk 166:542–544

Waite TA (1990) Effects of caching supplemental food on induced feather regeneration in wintering gray jays *Perisoreus canadensis*: a ptilochronology study. Ornis Scand 21(2):122–128. https://doi.org/10.2307/3676807

Zduniak P, Surmacki A, Erciyas-Yavuz K, Chudzińska M, Barałkiewicz D (2014) Are there different requirements for trace elements in eumelanin- and pheomelanin-based color production? A case study of two passerine species. Comp Biochem Physiol A 175:96–101. https://doi.org/10.1016/j.cbpa.2014.05.019