A peer-reviewed version of this preprint was published in PeerJ on 21 August 2018.

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Brandies PA, Grueber CE, Ivy JA, Hogg CJ, Belov K. 2018. Disentangling the mechanisms of mate choice in a captive koala population. PeerJ 6:e5438 https://doi.org/10.7717/peerj.5438

Disentangling the mechanisms of mate choice in a captive koala population

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Successful captive breeding programs are crucial to the long-term survival of threatened species. However, pair incompatibility limits sustainability of many captive populations. Understanding whether the drivers of this incompatibility are behavioural or genetic, or a combination of both, is crucial to improving breeding programs. We used twenty-eight years of pairing data from the San Diego Zoo koala colony, plus genetic analyses using both MHC-linked and non-MHC-linked microsatellite markers, to show that both behavioural and genetic determinants can influence mating success. Male age was reconfirmed to be a contributing factor to the likelihood of a pair copulating. Familiarity was also reconfirmed to increase the probability of a successful copulation. Our data provided evidence that females select mates based on MHC and genome-wide similarity. Male heterozygosity at class II MHC loci influenced both pre- and post-copulatory female choice. Genome-wide similarity and similarity at the MHCII DAB locus were also found to influence female choice at the post-copulatory level. Finally, certain MHC-linked alleles were associated with increased or decreased mating success. We predict that utilising a variety of behavioural and MHC-dependent mate choice mechanisms improves female fitness through increased reproductive success. This study highlights the complexity of mate choice mechanisms within a species and the importance of ascertaining mate choice mechanisms to improve the success of captive breeding programs.

Disentangling the mechanisms of mate choice in a captive koala population

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ABSTRACT

Successful captive breeding programs are crucial to the long-term survival of threatened species. However, pair incompatibility limits sustainability of many captive populations. Understanding whether the drivers of this incompatibility are behavioural or genetic, or a combination of both, is crucial to improving breeding programs. We used twenty-eight years of pairing data from the San Diego Zoo koala colony, plus genetic analyses using both MHC-linked and non-MHC-linked microsatellite markers, to show that both behavioural and genetic determinants can influence mating success. Male age was reconfirmed to be a contributing factor to the likelihood of a pair copulating. Familiarity was also reconfirmed to increase the probability of a successful copulation. Our data provided evidence that females select mates based on MHC and genome-wide similarity. Male heterozygosity at class II MHC loci influenced both pre- and post-copulatory female choice. Genome-wide similarity and similarity at the MHCII DAB locus were also found to influence female choice at the post-copulatory level. Finally, certain MHC-linked alleles were associated with increased or decreased mating success. We predict that utilising a variety of behavioural and MHC-dependent mate choice mechanisms improves female fitness through increased reproductive success. This study highlights the complexity of mate choice mechanisms within a species and the importance of ascertaining mate choice mechanisms to improve the success of captive breeding programs.

INTRODUCTION

Captive breeding programs contribute to species conservation and prevent extinction (Fa et al. 2011). The number of endangered and critically endangered species has been growing every year, largely due to human activities (IUCN 2016). At present, there are almost 25 000

threatened species on the IUCN red list and the need for effective captive breeding programs is more crucial than ever before (IUCN 2016). The primary goal of many captive breeding programs is to ensure that the long-term viability of a threatened population is maintained through ex-situ conservation of wild populations (Ballou et al. 2010). However, approximately 50% of captive populations are not sustainable: sufficient animals cannot be bred to retain the required levels of genetic diversity (Lees & Wilcken 2009).

Many captive breeding programs aim to minimise overall relatedness of animals and thereby maximise genetic diversity of a population, by pairing individuals for breeding that maintain equal genetic representations of founder lineages (Asa et al. 2011a). While this traditional strategy has been recognised and applied in captive facilities globally (Ballou & Lacy 1995), low breeding rates may occur due to mate incompatibility between individuals in prescribed pairs (Asa et al. 2011a; Lindburg & Fitch-Snyder 1994; Martin-Wintle et al. 2015; Quader 2005). New strategies that incorporate mate choice into conservation efforts are important for enhancing animal productivity and increasing the sustainability of captive populations (Asa et al. 2011a; Asa et al. 2011b; Lindburg & Fitch-Snyder 1994; Wedekind 2002).

Mate choice occurs as a result of non-random allocation of reproductive investment by individuals (Edward 2015; Paul 2002). Mate choice mechanisms can be pre-copulatory, whereby visual, chemical, acoustic or behavioural cues influence the likelihood of mating, and/or post-copulatory, whereby copulatory plugs, sperm destruction and other mechanisms alter insemination or fertilisation success (reviewed in Neff & Pitcher 2005; Paul 2002). Recent literature has also demonstrated the importance of the genetic determinants that underpin mate choice decisions in a wide range of species (for an overview of recent publications on this topic,

see Supplementary Table S1). There are currently three main, non-mutually exclusive hypotheses that can explain why the choosier sex (often females) selects mates based on genetic characteristics: A) quantity of alleles, B) genetic compatibility between mates, and C) advantage of particular alleles (reviewed in Kamiya et al. 2014; Setchell & Huchard 2010). Under the quantity of alleles hypothesis, females experience a fitness advantage by mating with males with greater heterozygosity, or those that carry the greatest number of alleles and hence the highest genetic diversity (Agbali et al. 2010; Kamiya et al. 2014; Penn & Potts 1999). Under the genetic compatibility hypothesis, females that mate with males who are genetically dissimilar, or with haplotypes that will best complement the females', experience a fitness advantage due either to certain combinations of haplotypes increasing offspring survival or to dissimilar matings resulting in offspring with higher genetic diversity (Neff & Pitcher 2005; Tregenza & Wedell 2000). Finally, the third hypothesis suggests females prefer males harboring particular alleles that provide offspring with greater immunity to parasites and/or infectious diseases, as often only one or a few alleles provide resistance to a specific pathogen (Bonneaud et al. 2005; Penn & Potts 1999).

The hypotheses described above may apply genome-wide or to specific loci, such as genes of the Major Histocompatibility Complex (MHC). MHC genes play a vital role in the vertebrate adaptive immune response as each MHC allele encodes a molecule that recognizes specific antigenic peptides and triggers the activation of T-cells (Balakrishnan & Adams 1995). Within the MHC gene family there are two main classes of molecules: class I molecules bind virus-derived peptides and stimulate cytotoxic T-cells, and class II molecules bind peptides from extracellular bacteria and larger parasites and stimulate other specific T-cells to stimulate the production of antibodies (Balakrishnan & Adams 1995; Milinski 2006). Therefore, having many

84 different MHC alleles increases the ability to respond to a larger range of pathogens (Penn &
85 Potts 1999). Additionally, species may also prefer to mate with individuals that show greater
86 genome-wide dissimilarity and/or heterozygosity to reduce inbreeding and maximize offspring
87 genetic diversity (Ferrandiz-Rovira et al. 2016; Kempenaers 2007). In consequence, numerous
88 studies have found evidence for one or more of the proposed hypotheses, considering both
89 genome-wide and MHC-dependent mate preferences (Table S1). However, little research
90 considers behavioural factors that may underlie mate choice decisions, in combination with
91 genetic determinants of mate choice.
92 In this study, we investigated the role of mate choice in the San Diego Zoo koala colony.
93 San Diego Zoo's breeding program commenced in 1981 and that facility continues to house the
94 largest koala colony outside of Australia. Despite increased pairing efforts (Fig. S1), the colony
95 has shown significant declines in copulation and breeding success over time (Fig. S2),
96 potentially due to reduced mate choice opportunities (Asa et al. 2011a). Familiarity and age have
97 previously been proposed to be important factors involved in koala mate choice in captivity
98 (Bercovitch et al. 2006), while evidence for size-mediated sexual selection in the koala has been
99 contradictory due to variable results (Bercovitch et al. 2006; William & Bercovitch 2011). The
100 vomeronasal organ of the koala is predicted to play a role in MHC-based olfactory
101 discrimination (Hegde 2003) and suggests a potential mechanism for this species to select mates
102 based on genetic characteristics in natural settings; however, it is currently unknown whether
103 genetic factors may also be influencing mate choice in captive koala populations. Our study aims
104 to 1) investigate behavioural factors that may be influencing mate choice in the San Diego Zoo
105 koala colony, using detailed pairing records and 2) test for evidence of the three mate choice
106 hypotheses (quantity of alleles, genetic compatibility and advantage of particular alleles) in

regard to both MHC-dependent (using MHC-linked microsatellites as a proxy for MHC variation) and genome-wide (using non-MHC-linked microsatellites as a proxy for genome-wide diversity) mating preferences. Determining how species make mate choice decisions, and the extent to which both behavioural and genetic factors influence breeding success in captive populations, will enable more effective captive breeding strategies and assist in improving the sustainability of captive breeding programs (Asa et al. 2011a; Quader 2005).

METHODS

Study Samples

Seventy koala DNA samples were provided by San Diego Zoo from banked samples (collected under San Diego Zoo Global IACUC protocols 10-008, 10-009, 11-029, 14-034) and shared with us for the purposes of this study. Detailed pairing records also were provided by the San Diego Zoo and studbook data were provided by the Association of Zoos & Aquariums North American Regional Studbook Keeper (Chris Hamlin, personal communication). These pairing records spanned 1984-2012 and contained mate choice data and breeding outcomes for every pairing (n = 964) at the zoo throughout this time. Breeding recommendations are reviewed annually and based predominately on a pairing strategy that aims to minimise kinship across the living population (Ballou & Lacy 1995). During pairing, estrous females are placed with males in enclosed cubicles for 5-10 minutes and mating behaviour is monitored throughout this time (Bercovitch et al. 2006). If the pair does not copulate, the female and male may be paired with other conspecifics, or the same pairing may be trialed again at a later time. This pairing process means both female and male koalas are often exposed to multiple individuals of the opposite sex within and between seasons.

Samples were available for individuals that spanned the breadth and depth of the 28 years of pairing data (Fig. S3); they accounted for ~50% of the individuals and pairings in the complete dataset (Table S2). Twenty-two sampled koalas were never paired for breeding and excluded from the final mate choice analyses. These koalas were genotyped during the study and included in all marker analyses (see Marker Analysis in the supplemental information) to maximise sample sizes where possible. All behavioural factors were analysed using the complete pairing dataset for increased power (Cohen 2013) (see Behavioural Determinants of Koala Mate Choice methods below).

Behavioural Determinants of Koala Mate Choice

We used generalised linear models (GLMs) in R v 3.4.0 (R Core Team 2017) to test for behavioural effects on mating success (including copulation, breeding and offspring success; see below). GLMs were performed with binomial distribution as follows: for each pairing event (n = 964) we modelled whether the pair successfully copulated (1) or did not copulate (0), with predictor variables including year of the pairing (to account for changes in the breeding program over time), age of the female, age of the male and the number of years the male and female had previously been paired together (as a measure of familiarity). Age^2 squared was also included as the relationship between age and mating success was not predicted to be linear (Rose 1991). The dataset was then subset into only those pairs that successfully copulated (n = 304) and the same predictor variables were modeled against whether each of these pairs successfully bred (produced offspring) (1) or did not breed (0). The dataset was then further subdivided into only those pairs that successfully bred (n = 134) and the same predictor variables were modeled

against whether those pairs produced offspring that survived more than one year (1) or did not survive more than one year (0).

Age difference between the male and female was found to show a moderate negative correlation with female age, and a strong positive correlation with male age, and was therefore not included as a predictor variable (Table S3). Male body mass was also not included as a predictor as it has previously been shown to correlate strongly with male age (Tobey et al. 2006), and body mass data for the current study samples was unavailable. Although some pairs were repeated in multiple years, Pair ID was not included as a random factor due to majority of pairs (60%) only being represented in one year of the dataset (Fig. S4) (models with Pair ID fitted as a random intercept did not converge). Variance Inflation Factors (VIFs; Belsley et al. 1980) were calculated for the remaining predictor variables to ensure there were no adverse effects of multicollinearity. All VIFs were < 2 and so year, female age, male age and familiarity were included in the same model (Belsley et al. 1980). Model predictors were standardised by subtracting the mean and dividing by two standard deviations (following Gelman 2008) to facilitate inference of regression coefficients within and between models (Schielzeth et al. 2010). Model fitted values were back-transformed onto the natural scale for plotting and interpretation.

MHC Genotyping

The use of mulitple loci is preferable when testing MHC-dependent mate choice associations (Kamiya et al. 2014), but current MHC typing techniques (such as gene sequencing, single strand conformation polymorphism analysis, denaturing gradient gel electrophoresis or reference strand-mediated conformation analysis) are impractical due to the large numbers of duplicated MHC loci throughout marsupial genomes (Belov et al. 2013; Nei et al. 1997). We

therefore used MHC-linked microsatellites to quantify diversity at MHC loci. Gene sequences for all classical MHC class II genes previously characterised (excluding any putative pseudogenes) (Koala Genome Consortium Submitted), with 10 kb of flanking sequence, were extracted from koala genome scaffolds accessible through KoalaBASE (Koala Genome Consortium 2017; Priyam et al. 2015) (Table S4). Classical class II MHC genes were chosen based on their prominent mate choice effects in mammals (Table S1). RepeatMasker (Smit et al. 2013-2015) was used to identify microsatellite sequences < 10 kb away from the MHC genes (Cheng et al. 2009b). Candidate microsatellite sequences (PhciDBB001M3, PhciDCBM1 and MHCIIDAB001M1) were selected based on minimal interruptions to the repeat sequence and low proximity to other repeat regions. These microsatellites were linked to genes of the DB, DC and DA families respectively, allowing us to incorporate a representative for each classical marsupial MHCII family (Belov et al. 2006; Belov et al. 2004). The repeat motifs for each microsatellite were $(TG)_{13}$, $(GA)_{28}$ and $(AC)_{29}$ respectively. We extracted these microsatellite sequences with 300 bp of flanking sequence and designed PCR primers using Oligo 7 (Rychlik 2007). Primer sequences were then used in a BLAST search (Koala Genome Consortium 2017; Priyam et al. 2015) against the koala genome to ensure specificity and prevent amplification of non-target sequences. Primer sequences used to amplify the three microsatellites were as follows (CAG tags (Schable et al. 2002) in italics): PhciDBB001M3 F: *CAGTCGGGCGTCATCA*TTCTCTTGTCCTTCTTGTGTC, R:TTCTCCCTACAAAGATGATCC; PhciDCBM1 F:*CAGTCGGGCGTCATCA*AGTCTGGTGTCATTAGCAATAGG, R:CTGAATGAGGCAAGGGAGAG; MHCIIDAB001M1

F:ACACTACTTCCCTGAATCTGAC,

R:*CAGTCGGGCGTCATCA*TACAGTGTTACTTCATGCAGAG.

All primers were initially screened for polymorphism (see Initial Primer Screening and Optimisation Methods in the supplemental information) before genotyping the study population at these markers. PCRs were carried out using Qiagen Type-it Microsatellite PCR Kit with a modified total reaction size of 10 µL and the following modified primer concentrations: 0.06 µM tagged primer, 0.6 µM untagged primer and 0.6 µM 6-FAM labelled CAG tag. Thermocycling conditions followed a protocol of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 90 s and 72°C for 30 s with a final extension of 60°C for 30 min. Capillary electrophoresis was undertaken at the Australian Genome Research Facility (AGRF) using MCLAB DSMO-100 Orange Size Standard. Alleles were manually called using GeneMarker (Hulce et al. 2011). Controls included a negative control whereby water instead of DNA was added to the PCR reaction, and a positive control using DNA from a koala that was successfully genotyped during the initial primer screening.

Genotyping of Non-MHC-linked Microsatellites

Koalas were genotyped at a further 15 microsatellite loci, not known to be linked to MHC, using primers from previous studies (Cristescu et al. 2009; Dennison et al. 2017; Houlden et al. 1996) (Table S5). The locations of each microsatellite was confirmed using the NCBI koala assembly browser (Kitts et al. 2015). All microsatellites were located on scaffolds not containing any MHC genes, and most were >10 kb away from any genes (Table S5). Seven markers (Pcin05, Pcin08, Pcin11, Pcin20, Pcin21, Pcin22 and Pcin23) were split into 3 multiplexes using a fluorescently labelled (6-FAM) CAG-tag (Schable et al. 2002) (Table S5). PCRs were carried

out using the Qiagen Type-it Microsatellite PCR Kit and a fluorescently labelled (6-FAM) CAG-tag (as above).

PCR for markers Pcv31, Pcv25.2, Pcv30 and Pcv25.1 were carried out using a fluorescently labelled (HEX or 6-FAM) CAG-tag. Amplification was performed in a 10 uL reaction volume containing 1X PCR buffer, 2.5 mM MgCl, 0.2 mM dNTPs, 0.2 uM forward primer, 0.6 uM reverse primer, 0.6 uM CAG-tag and 0.5 U taq. Thermocycling conditions followed a protocol of 5 min at 95°C, then 25 cycles of 30 s at 94°C, 45 s at 55˚C and 30 s at 72°C, followed by 8 cycles of 30 s at 94˚C, 45 s at 53˚C and 45 s at 72˚C with a final extension of 72°C for 30 min. Amplification of the remaining 4 markers (Pcv24.2, Pcv26, Phc13, Phc11) was carried out with a fluorescently labelled forward primer (HEX or 6-FAM) using the same PCR mix (excluding the M13 tail). Thermocycling conditions followed a protocol of 5 min at 95°C, then 20 cycles of 30 s at 94°C, 45 s at 70˚C and 45 s at 72°C, followed by 15 cycles of 30 s at 94˚C, 45 s at the annealing temperature and 45 s at 72˚C with a final extension of 72°C for 10 min. Annealing temperature was 55˚C, except for Phc11 and Phc13 where the annealing temperature was 50˚C. Samples were genotyped on an ABI 3130xl using an internal GeneScan 500 ROX size standard and alleles were automatically called then manually checked using GeneMapper (ABI).

Microsatellite Diversity

Approximately 20% of the koalas were re-genotyped using the same methods to determine genotyping error rate. Tests for evidence of null alleles, deviation from Hardy-Weinberg equilibrium, and linkage disequilibrium were performed to ensure all of the non-MHC and MHC markers were suitable for use in the final statistical analysis (methods and results for

these analyses are provided in Marker Analysis in the supplemental information and Table S6). Standardised heterozygosity (H_s) was calculated as a measure of individual multilocus heterozygosity at both the non-MHC and MHC markers using the Rhh package (Alho et al. 2010) in R. We chose this method, as H_s gives equal weighting for all loci examined despite the differences in the number and frequency of alleles present across the markers used (Aparicio et al. 2006; Coltman et al. 1999). A Spearman's rank correlation between standardised heterozygosity at MHC-linked markers and standardised heterozygosity at non-MHC markers was also performed to test whether MHC-associated mate choice findings were correlated with (or by-products of) genome-wide variation (following Ferrandiz-Rovira et al. 2016).

Statistical Analysis

Generalised linear models performed in R were used to test the three mate choice hypotheses. All GLMs were performed with a binomial distribution as follows: "copulation success" was the number of successful copulations (binomial numerator: successes) out of the total number of pairings (binomial denominator: trials); "breeding success" was the number of offspring (successes) produced from successful copulations (trials); and "offspring success" was the number of offspring that survived more than one year (successes) out of the total offspring produced (trials). In each model, the predictor variables were as described in the sections that follow (Male Heterozygosity and Pair Similarity). Multicollinearity of predictor variables in all models was checked by calculating VIFs, and model predictors were standardised to facilitate inference across predictors, as described above.

Quantity of Alleles

To test whether mating success was influenced by genome-wide quantity of alleles, male standardised heterozygosity (H_s) across the fifteen non-MHC markers was modelled as the predictor, with copulation, breeding and offspring success for each male as separate response variables. To determine whether quantity of alleles at MHC loci influenced mating success, standardised heterozygosity (H_s) across the three MHC-linked loci was modelled against the three response variables. We also tested whether heterozygosity at the microsatellites linked to the DA, DB and DC loci (coded 1/0 for heterozygote/homozygote at each locus) influenced each of the three response variables. Since year of first pairing was found to be highly correlated with year ($\rho = 0.98$, $p = < 0.01$), year of first pairing for each male was added as an additional continuous fixed predictor to all models to account for changes in the breeding program over time. For models in which copulation success was the response, male age at first pairing was included as a predictor to account for the influence of male age on copulation success (see Results). Familiarity was not included as a predictor in these models as all males were paired with multiple females.

Genetic Compatibility

To test whether genome-wide genetic compatibility influenced mating success, we calculated the molecular coancestory (allele sharing) of each pair as a measure of similarity at non-MHC loci in MolKin v 2.0 (Gutiérrez et al. 2005), and modelled molecular coancestory as the predictor variable against copulation, breeding and offspring success. Molecular coancestory was used, rather than traditional relatedness measures, because meaningful estimates of allele frequencies are difficult to calculate for captive populations that are managed using non-random mating strategies and when samples are distributed across the depth of an overlapping,

289 generational pedigree (Ivy et al. 2016). We tested whether genetic compatibility at MHC loci
290 influences mating success by calculating MHC similarity for each unique breeding pair across
291 the three MHC-linked loci using Wetton's formula (Parkin et al. 1987): $D_{AB} = 2F_{AB} / (F_A + F_B)$;
292 where, F_{AB} is the total number of unique MHC-linked microsatellite alleles shared by a male (A)
293 and a female (B) across the typed loci; and F_A and F_B are, respectively, the total number of
294 alleles of the male (A) and female (B) (Parkin et al. 1987). This formula was chosen as it is
295 commonly used to determine similarity at MHC loci (Huchard et al. 2010; Olsson et al. 2003)
296 and enabled us to also assess each MHC locus (DA, DB and DC) separately (other similarity
297 estimators rely on multi-locus data). MHC similarity (either overall, or at each locus) was then
298 modelled against the copulation, breeding and offspring success of each pair. To account for
299 changes in the breeding program over time, the first year of pairing for each pair was added to all
300 models as an additional predictor. For each breeding pair, the male's age at first pairing and the
301 total number of years paired together were also included as additional predictors for models
302 where copulation success was the response. This was done to account for the influence of male
303 age and familiarity on copulation success respectively (see Results).
304
305 **Advantage of Particular Alleles**
306 Under the advantage of the particular alleles hypothesis, the null hypothesis that specific
307 MHC alleles do not influence mating success was tested by coding each male with a 1/0
308 predictor indicating the presence or absence, respectively, of each allele of the three MHC-linked
309 loci (Sepil et al. 2012) and modelling these predictors separately against each of the response
310 variables of copulation, breeding and offspring success. To account for any effect of year or male
311 age, year of first pairing was included as a predictor in every model and male's age at first

pairing was included as an additional predictor in models with copulation success as the predictor. A "base" model, which excluded allelic information, was also fitted for each response variable across the three loci. For each response variable, all models were ranked by AIC_C (Burnham & Anderson 2002) to determine the relative level of support for each allele as a predictor of mating success. Models that were highly ranked (i.e. ≥ 2 AIC_C above the next best model and the base model) were interpreted as providing strong evidence that the presence or absence (if a positive or negative regression slope, respectively) of a given allele had an effect on the corresponding response variable (Sepil et al. 2012).

RESULTS

Behavioural Determinants of Koala Mate Choice

Male age was found to have a significant effect on copulation success (Table 1). Expected copulation success rates increased from ~20% when males were at their youngest (2 years old), to 40% when males reached 12 years of age, and decreased to below 35% when males were 17 years or older (fitted values are taken from the regression model in Table 1). Copulation success was also found to increase significantly with increasing familiarity between pairs (Table 1). Dyads that had previously been paired together for 5 years or more had expected copulation success rates above 50% (95% CI = 0.36, 0.65), compared to the 34% success rate of dyads that had never previously been paired together (95% CI = 0.28, 0.41; fitted values are taken from the regression model in Table 1). No association was found between female age and mating success (Table 1). None of these factors were found to influence breeding or offspring success, and could not explain the strong declines in copulation and breeding success of pairs throughout the years of the breeding program (Table 1).

335

336 *Microsatellite Genotyping*

337 All koalas were genotyped at the three MHC markers and > 75% of the study population
338 was genotyped at 13 or more of the non-MHC loci (Table S6). Genotyping error rate, based on
339 re-genotyping of ~20% of individuals, was very low (0.53%). Standardised heterozygosity (H_s)
340 for the MHC markers ranged from 0.43 (more homozygous) to 1.3 (more heterozygous) (Table
341 S6). Non-MHC marker standardised heterozygosity (H_s) ranged from 0.35 (more homozygous)
342 to 1.62 (more heterozygous). Standardised heterozygosity at MHC-linked loci was not correlated
343 to standardised heterozygosity at non-MHC loci (n = 70 koalas, ρ = -0.06, 95% CI = -0.30, 0.19,
344 p = 0.614).

345

346 **Quantity of Alleles**

347 For the MHC-linked loci there was a negative relationship between male standardized
348 heterozygosity and copulation success (Table 2A). Examining each MHC locus separately
349 suggested that the overall trend may result primarily from heterozygosity at the MHCII DAB
350 locus (Table 2B). Amongst those males that successfully copulated, males with higher overall
351 MHC heterozygosity showed significantly greater breeding success rates than less heterozygous
352 males (Table 2A). For example, our models predict that males that were heterozygous at all three
353 MHC-linked loci had expected copulation success rates of 22% (95% CI = 0.15, 0.31) and
354 breeding success rates of 49% (95% CI = 0.29, 0.68), whereas males that were homozygous at all
355 three MHC-linked loci had expected copulation success rates of 56% (95% CI = 0.34, 0.77) and
356 breeding success rates of 9% (95% CI = 0.02, 0.32; fitted values are taken from the regression
357 models in Table 2). No association was found between offspring survival and heterozygosity at

MHC loci (Table 2). For the non-MHC-linked loci, male heterozygosity did not show a significant effect on copulation, breeding nor offspring success (Table 2C). Year and age were found to have a significant influence on mating success in line with our findings based on the larger demographic dataset (Table 2).

Pair Compatibility

Similarity at MHC-linked loci was not found to have a significant effect on copulation success (Table 3); however, pairs with a higher similarity at the MHC-linked DAB locus were found to have a significantly greater breeding success rate than more dissimilar pairs (Table 3B). For example, our models predicted that pairs that share one allele or more at the MHCII DAB locus would have an expected breeding success rate of 40% or higher (95% CI = 0.24, 0.56), compared to pairs that shared no alleles, which would have an expected breeding success rate of 20% (95% CI = 0.13, 0.30; fitted values are taken from the regression model in Table 3). There were no significant effects of MHC similarity on offspring success (Table 3). Genome-wide similarity was not found to have a significant effect on copulation nor offspring success; however, pairs with a higher similarity at non-MHC loci were found to have significantly greater breeding success rates than more dissimilar pairs (Table 3C). For example, expected breeding success rates increased from 15% (95% CI = 0.08, 0.27) to 57% (95% CI = 0.24, 0.84) as genome-wide similarity estimates increased from 0.2 (low allele sharing at non-MHC loci between pairs) to 0.6 (high allele sharing at non-MHC loci between pairs) respectively (fitted values are taken from the regression model in Table 3). Year, familiarity and male age were all found to have the same significant effects on mating success as discussed above (Table 3).

Advantage of Particular Alleles

Copulation success rates were higher in males that did not carry the DCB226 or DCB254 allele than males that did carry either of these alleles (Table 4). Conversely, males that carried the DCB266 allele were more likely to produce offspring than males without the allele (Table 4). Males that carried the DBB297 allele also showed higher breeding success rates than males with other alleles at this locus, while males that carried the DAB289 allele showed reduced breeding success rates relative to males that did not carry the allele (Table 4). No particular alleles were found to influence offspring success (Table 4). While some of these models appear to provide strong evidence for the influence of certain alleles on mating success, cautious interpretation of these findings is warranted as the reliability of these models may be limited due to small sample sizes and small subject:predictor ratios (Table 4).

DISCUSSION

Ours is the first study to examine behavior, as well as both genome-wide and MHC-dependent mate choice preferences, at multiple stages of the mating process in a captive koala population. We reconfirmed that both age and familiarity were determinants of mating success in this species. There was evidence of genome-wide mate preferences as well as pre-copulatory and post-copulatory MHC-dependent mate choice under all three mate choice hypotheses, A) quantity of MHC alleles; B) genetic compatibility between mates; and C) advantage of particular alleles (hypotheses reviewed in Kamiya et al. 2014; Setchell & Huchard 2010). These results suggest that koalas use a combination of behavioural and MHC-dependent mate choice mechanisms to select mates of the highest genetic quality, and to optimise both the quantity and combination of alleles in their offspring.

404

405 *Behavioural Determinants of Koala Mate Choice*

406 In line with earlier studies of koala mate choice (Bercovitch et al. 2006), our analysis
407 found that koala copulation success is significantly influenced by male age and/or the age
408 difference between males and females. Many empirical studies in other species have also
409 suggested that females may prefer to mate with older males, likely due to older males being of a
410 higher genetic quality through viability selection (Manning 1985; Trivers 1972). In koala, male
411 size, bellowing and sternal scent secretions have been found to convey age-related information
412 (Charlton et al. 2012; Salamon & Davies 1998; Tobey et al. 2006), and so it is predicted that
413 females may use visual, auditory and chemical cues to select mates based on age (Bercovitch et
414 al. 2006). Our study provides additional evidence that male age influences koala mate choice in
415 captivity, although the precise chemical and auditory mechanisms by which females receive and
416 utilise this information remain unclear (Ellis et al. 2015; Tobey et al. 2009).

417 In addition to age, we also found that familiarity may promote copulation success in
418 captive koala populations. Mate choice studies in other mammals have shown that females may
419 show a preference for more familiar males (Roberts & Gosling 2004) and that mating with
420 familiar males can lead to increased reproductive success (Martin & Shepherdson 2012). A
421 preference for familiar males often arise in territorial scent-marking species, as females are more
422 likely to encounter scent marks of locally territorial males and as a result select these males due
423 to their ability to defend a territory (Rich & Hurst 1998). Although koalas are a territorial scent-
424 marking species (Allen et al. 2010), female koalas do not show a preference for locally territorial
425 males in the wild (Hale & Carrick 2002). It is plausible that the familiarity trend in the current
426 study may have been driven by pairing previously successful pairs together in subsequent years,

427 although most (60%) of the pairings in our dataset were from first-time pairings (see Methods).
428 Further research, directly examining the role of familiarity in koala mate choice, is needed to
429 confirm whether familiarity is important in koala mate choice both in captivity and in the wild.
430
431 *Genetic Determinants of Koala Mate Choice*
432 Previous research suggests that females are often more attracted to heterozygous males,
433 and heterozygosity has been linked to numerous advantages such as greater sexual
434 ornamentation, mating success and overall reproductive success (reviewed in Kempenaers 2007).
435 Despite these advantages, genome-wide heterozygosity was not found to influence mating
436 success in our analysis of captive koalas. Some species have also been found to display a
437 preference for dissimilar individuals, which may reduce inbreeding and increase genetic diversity
438 of offspring (Ferrandiz-Rovira et al. 2016; Kempenaers 2007). In contrast, we found a positive
439 association between genome-wide similarity and breeding success, suggesting female koalas are
440 more likely to produce offspring with males that are more genetically similar overall. A recent
441 review highlighted how mating with similar individuals can allow populations to adapt more
442 quickly to virulent diseases and parasites (Campbell et al. 2017). Assortative mate preferences
443 may therefore help protect koala populations from threatening infectious diseases such as
444 chlamydia and koala retrovirus, and should be examined further. We note that although similar
445 numbers of neutral microsatellite markers have been used in recent studies to examine genome-
446 wide mating preferences (Ferrandiz-Rovira et al. 2016; Huchard et al. 2013), estimates of
447 genome-wide diversity based on 15 microsatellites may not be sufficient (Miller et al. 2014;
448 Takezaki & Nei 1996) and larger numbers of markers should be employed in future studies to
449 provide more accurate measures of genome-wide diversity.

In addition to genome-wide mating preferences, many species have been found to select mates based on MHC (Table S1). Consistent with these studies, we found that mating success showed a significantly association with male heterozygosity and pair similarity at MHC loci, as well as the presence or absence of particular MHC alleles. In contrast to the quantity of alleles hypothesis, males that were less heterozygous at MHC-linked loci showed a greater rate of copulation success, indicating that female koalas prefer to copulate with males that have fewer alleles at MHC loci, particularly at DAB loci. Interestingly, among those males that did copulate, breeding success was higher for more heterozygous males. This suggests that females are more likely to produce offspring when breeding with males of higher heterozygosity, than when breeding with males of lower heterozygosity. The standardised slopes of the trends at each stage were of similar magnitude (Table 2), suggesting that the effect of heterozygosity is similar at both mate choice stages. Taken together, these results reflect differences in the pre-copulatory and post-copulatory MHC-dependent choice mechanisms in the koala.

Previous research has shown that vertebrate females can select sperm based on heterozygosity or diversity at MHC loci (Wedekind 2002; Winternitz et al. 2013). Males that are heterozygous at MHC loci also show significantly greater fertilisation success relative to homozygous males (Skarstein et al. 2005). It appears that in the koala, males with low heterozygosity at MHC loci overall (particularly at DAB loci) have a higher probability of copulating; however, more heterozygous males may experience a fertilisation advantage, so that their copulations are more likely to result in the production of offspring. While the benefits of breeding with heterozygous males can be explained by the increased antigenic peptide repertoire and immunocompetence of heterozygotes (Kamiya et al. 2014; Landry et al. 2001), we are unaware of any other reports that less-heterozygous males have a higher copulation success rate

473 in other species. Further work should investigate whether this unexpected relationship may be
474 driven by an unmeasured male trait that is correlated with MHC heterozygosity.
475 Contrary to many previous findings under the genetic compatibility hypothesis (Table
476 S1), captive koala pairs that were more similar at the MHC DAB-linked locus were found to
477 have greater breeding success than less similar pairs. In our analysis, female koalas were more
478 likely to produce offspring with males that share alleles at DAB loci, which would be consistent
479 with a greater reliance on post-copulatory MHC-dependent mechanisms of mate choice in this
480 species. Numerous studies have found that females select sperm based on the genetic
481 dissimilarity of mates (Olsson et al. 1996; Thuman & Griffith 2005), particularly at MHC loci
482 (Løvlie et al. 2013; Schwensow et al. 2008; Yeates et al. 2009). Whilst a preference for MHC-
483 dissimilar mates is predicted to be more common among species, in order to maximize MHC
484 diversity of offspring (Kamiya et al. 2014; Landry et al. 2001; Milinski 2006; Tregenza &
485 Wedell 2000), a preference for mates that are more similar at MHC loci may evolve in response
486 to disadvantages associated with mating with individuals that are too dissimilar, including
487 increased risk of autoimmune disorders due to suboptimal T-cell selection (Kaufman 1999;
488 Kaufman et al. 1995), reduced recognition of foreign peptides due to T-cell loss (Nowak et al.
489 1992; Vidovi & Matzinger 1988), and disruption of co-adapted gene complexes (Hendry et al.
490 2000).
491 Studies have also shown that, in some circumstances, carrying multiple copies of the
492 same MHC allele allows for higher disease resistance (Grimholt et al. 2003; Nuismer et al.
493 2008). However, MHC assortative mating may make populations more vulnerable to future
494 disease outbreaks or other stochastic events (Campbell et al. 2017). Therefore, we suggest that
495 female koalas may not solely choose more-similar mates but may rather optimise the quantity

and combination of MHC alleles in the offspring (see also Milinski 2006). A similarly complex mate choice mechanism has been demonstrated in sticklebacks (*Gasterosteus aculeatus*), whereby females prefer to mate with males with genotypes that, when combined with their own MHC alleles, will produce offspring with an optimal number of alleles and provide the highest possible resistance against common parasites (Milinski 2003; Reusch et al. 2001). Further analyses that examine numbers and combinations of MHC alleles in offspring with reference to the parents' MHC are required to confirm whether the same mechanism exists in the koala.

In line with the advantage of the particular alleles hypothesis, we found that the presence of certain MHC-linked microsatellite alleles was associated with increased or decreased mating success, whilst some alleles showed no association. Since the MHC-linked microsatellites are found in non-coding regions of the genome (and likely have no functional implications), this finding suggests that females are selecting for and/or against males that carry the respective MHC alleles. Preferences for certain MHC alleles or genotypes have been found in multiple species, and is believed to be due to certain alleles being associated with resistance or susceptibility to pathogens (Cutrera et al. 2012; Eizaguirre et al. 2009). Class II MHC molecules are responsible for the presentation of bacterial antigens to the immune system (Balakrishnan & Adams 1995; Milinski 2006). Consequently, MHCII alleles of the koala have previously been associated with resistance and susceptibility to chlamydia (Lau et al. 2012). Certain MHC alleles have also been found to be associated with increased fertilisation success in other species (Skarstein et al. 2005). Female preferences for males carrying certain MHC alleles therefore increase reproductive success and provide offspring with optimal immunity against common pathogens, maximizing their chances of survival (Milinski 2006).

Overall, our findings allow us to hypothesize that particular MHC alleles, together with heterozygosity and similarity at MHC loci, work together to drive mate choice in captive koalas. This complex combination of MHC-dependent mechanisms is predicted to optimise both the quantity and combination of MHC alleles in the offspring, thereby increasing offspring survival (Milinski 2006). Mate choice has been found to influence offspring viability in a variety of species, particularly when mating preferences are MHC-dependent (Agbali et al. 2010; Von Schantz et al. 1996). Our study did not show an association between offspring survival and mate choice preferences, however, data on offspring survival was only present for koala pairings that produced offspring (n = 13 males, 26 pairs, 28 offspring in total) and so sample sizes were small. Nevertheless, if observed mating preferences produce offspring with optimal MHC, any successful matings may have resulted in offspring with optimal immunity, and any early offspring deaths might be more likely to result from factors unrelated to MHC or immunity in general (such as infection). This hypothesis could be tested by closer examination of the cause of joey losses (and whether these are associated with offspring immunity).

By using MHC-linked microsatellites we were able to examine three families of MHC loci simultaneously. The majority of MHC-dependent mate choice studies in the current literature often only examine a single locus due to the limitations of MHC typing techniques (Kamiya et al. 2014). Our data indicate that some loci may play a larger role in mate choice than others, and different loci may act in different ways, further indicating the importance of examining multiple MHC loci. Huchard et al. (2013) found that female grey mouse lemurs (*Microcebus murinus*) chose males based on a particular MHCII locus under stronger diversifying selection. Similarly, DAB loci in the koala have previously been found to be under stronger selection than other MHCII loci (Abts et al. 2015; Lau et al. 2013), which may explain

541 the strong effect we found this locus to have on koala mate choice. Multi-locus approaches are
542 vital in gaining a holistic understanding of MHC-dependent mate choice mechanisms (Kamiya et
543 al. 2014) and can be easily achieved using MHC-linked microsatellite markers. Although
544 numerous studies have confirmed MHC-linked microsatellite markers as a proxy for MHC
545 diversity in other species (Cheng & Belov 2012; Cheng et al. 2009a; Crouau-Roy et al. 1996),
546 this association needs to be confirmed in the koala.
547
548 *Conclusions*
549 In conclusion, pair incompatibility is an important contributing factor for why many
550 captive breeding programs are failing to reach program goals (Lees & Wilcken 2009). We found
551 a significant decrease in the copulation and breeding success of our study population, indicating
552 a potential risk to future sustainability. The age of males and familiarity between pairs were
553 found to play some role in mate choice. We also found evidence that genome-wide similarity and
554 MHC-diversity were associated with mating success, and mate choice mechanisms may
555 consequently be contributing to reduced copulation and breeding success rates. Our findings
556 have shown the importance of examining both the behavioural and genetic determinants of mate
557 choice in captive populations, and will help aid future pairing recommendations in captive
558 facilities. This study therefore has important implications, not only for the management of
559 captive koalas, but for all conservation initiatives for threatened species where breeding is
560 managed.
561
562
563 *Acknowledgements:*

We thank San Diego Zoo Global for providing DNA samples, studbook data and pairing records.

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Table 1 (on next page)

Generalised linear models of the relationships between year, age, familiarity and three measures of mating success.

Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Table 1. Generalised linear models of the relationships between year, age, familiarity and three measures of mating success. Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Response Variable	n	Predictor Variable	Slope ± SE	z value	P
Copulation Success	964	**Year**	**-0.97 ± 0.16**	**-6.083**	**< 0.001**
		Female Age^{2*}	-0.53 ± 0.29	-1.866	0.062
		Female Age	-0.30 ± 0.18	-1.728	0.084
		Male Age^{2*}	**-0.67 ± 0.29**	**-2.266**	**0.023**
		Male Age	**0.54 ± 0.20**	**2.713**	**0.007**
		Familiarity	**0.34 ± 0.17**	**2.031**	**0.042**
Breeding Success	304	**Year**	**-1.07 ± 0.29**	**-3.729**	**< 0.001**
		Female Age^{2*}	0.10 ± 0.41	0.244	0.807
		Female Age	-0.3 ± 0.30	-1.024	0.306
		Male Age^{2*}	0.37 ± 0.46	0.802	0.422
		Male Age	-0.30 ± 0.31	-0.963	0.335
		Familiarity	0.05 ± 0.29	0.185	0.853
Offspring Success	134	**Year**	**0.89 ± 0.45**	**2.007**	**0.045**
		Female Age^{2*}	-0.70 ± 0.62	-1.127	0.260
		Female Age	-0.31 ± 0.44	-0.713	0.476
		Male Age^{2*}	-0.02 ± 0.64	-0.038	0.970
		Male Age	-0.33 ± 0.50	-0.653	0.514
		Familiarity	0.64 ± 0.49	1.299	0.194

* Squared term used to create a polynomial model as the relationship between age and mating success was not predicted to be linear

Bolded predictors show coefficients that are statistically different from 0 at the .05 alpha level

Table 2 (on next page)

Generalised linear models of the relationship between male heterozygosity and mating success. > l,? G[l"

Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Table 2. Generalised linear models of the relationship between male heterozygosity and mating success. Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Response Variable	n	Predictor Variable*	Slope ± SE	z-value	P
A. Overall MHC Heterozygosity					
Copulation Success	21	**Intercept**	**-0.79 ± 0.102**	**-7.74**	**< 0.001**
		Year	**-1.32 ± 0.23**	**-5.77**	**< 0.001**
		Age	**0.46 ± 0.169**	**2.71**	**0.007**
		H_s	**-0.51 ± 0.217**	**-2.35**	**0.019**
Breeding Success	17	**Intercept**	**-0.77 ± 0.201**	**-3.83**	**< 0.001**
		Year	**-1.51 ± 0.414**	**-3.65**	**< 0.001**
		H_s	**0.79 ± 0.372**	**2.13**	**0.034**
Offspring Success	13	**Intercept**	**1.35 ± 0.493**	**2.73**	**0.006**
		Year	1.68 ± 0.925	1.82	0.069
		H_s	-0.76 ± 0.687	-1.10	0.270
B. Individual MHC Heterozygosity					
Copulation Success	21	**Intercept**	**-0.79 ± 0.102**	**-7.77**	**< 0.001**
		Year	**-1.48 ± 0.279**	**-5.30**	**< 0.001**
		Age	**0.46 ± 0.169**	**2.75**	**0.006**
		DBB Heterozygosity	-0.31 ±	-1.32	0.187

Response Variable	n	Predictor Variable*	Slope ± SE	z-value	P
		(6,15)	0.239		
		DCB Heterozygosity (3,18)	-0.37 ± 0.341	-1.10	0.273
		DAB Heterozygosity (4, 17)	**-0.73 ± 0.346**	**-2.10**	**0.036**
Breeding Success	17	**Intercept**	**-0.79 ± 0.211**	**-3.76**	**< 0.001**
		Year	**-1.4 ± 0.489**	**-2.86**	**0.004**
		DBB Heterozygosity (5, 12)	0.55 ± 0.379	1.44	0.149
		DCB Heterozygosity (2, 15)	0.82 ± 0.563	1.45	0.147
		DAB Heterozygosity (3, 14)	0.97 ± 0.846	1.14	0.253
Offspring Success	13	**Intercept**	**1.26 ± 0.402**	**3.13**	**0.002**
		Year	**1.83 ± 0.881**	**2.07**	**0.038**
		DBB Heterozygosity (4, 12)	-1.05 ± 0.625	-1.69	0.092
		DCB Heterozygosity (1, 12)	NA	NA	NA
		DAB Heterozygosity (1, 12)	NA	NA	NA
C. Genome-wide Heterozygosity					
Copulation Success	21	**Intercept**	**-0.91 ± 0.094**	**-9.75**	**<0.001**
		Year	**-1.69 ± 0.334**	**-5.07**	**<0.001**
		Age	**0.37 ±**	**2.28**	**0.023**

Response Variable	n	Predictor Variable*	Slope ± SE	z-value	P
			0.163		
		H_s	-0.47 ± 0.324	-1.45	0.146
Breeding Success	17	**Intercept**	**-0.59 ± 0.178**	**-3.32**	**0.001**
		Year	**-1 ± 0.495**	**-2.03**	**0.042**
		H_s	0.62 ± 0.512	1.22	0.224
Offspring Success	13	**Intercept**	**0.98 ± 0.353**	**2.79**	**0.005**
		Year	1.26 ± 0.792	1.59	0.112
		H_s	0.24 ± 0.887	0.27	0.788

* Numbers in parentheses indicate the number of homozygotes and heterozygotes respectively. Any loci with <2 homozygotes were not fitted, but are shown in the table for completeness (denoted “NA”).
Bolded predictors show coefficients that are statistically different from 0 at the .05 alpha level

Table 3 (on next page)

Generalised linear models of the relationship between pair similarity and mating success.

Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Table 3. Generalised linear models of the relationship between pair similarity and mating success. Predictor variables were standardised by subtracting the mean and dividing by two standard deviations (see Methods).

Response Variable	n	Predictor Variable	Slope ± SE	z-value	P
A. Overall MHC Similarity					
Copulation Success	89	**Intercept**	**-1.16 ± 0.132**	**-8.79**	**< 0.001**
		Year	**-0.6 ± 0.242**	**-2.46**	**0.014**
		Familiarity	**0.46 ± 0.212**	**2.17**	**0.030**
		Male Age	0.44 ± 0.241	1.84	0.066
		MHC Similarity	0.32 ± 0.201	1.59	0.112
Breeding Success	53	**Intercept**	**-1.02 ± 0.223**	**-4.59**	**< 0.001**
		Year	**-1.31 ± 0.427**	**-3.08**	**0.002**
		MHC Similarity	0.74 ± 0.382	1.93	0.054
Offspring Success	26	**Intercept**	**1.42 ± 0.542**	**2.62**	**0.009**
		Year	1.4 ± 0.847	1.66	0.098
		MHC Similarity	0.4 ± 0.701	0.56	0.572
B. Individual MHC Similarity					
Copulation Success	89	**Intercept**	**-1.16 ± 0.133**	**-8.72**	**< 0.001**
		Year	**-0.71 ± 0.255**	**-2.77**	**0.006**
		Familiarity	**0.48 ± 0.212**	**2.28**	**0.022**
		Male Age	**0.51 ± 0.245**	**2.08**	**0.038**
		DBB Similarity	0.44 ± 0.227	1.91	0.056
		DCB Similarity	0.11 ± 0.24	0.45	0.652
		DAB Similarity	-0.03 ± 0.243	-0.13	0.900
Breeding Success	53	**Intercept**	**-0.98 ± 0.225**	**-4.36**	**< 0.001**
		Year	**-1.14 ± 0.463**	**-2.46**	**0.014**

Response Variable	n	Predictor Variable	Slope ± SE	z-value	P
		DBB Similarity	0.61 ± 0.434	1.41	0.158
		DCB Similarity	-0.13 ± 0.468	-0.29	0.773
		DAB Similarity	**0.99 ± 0.452**	**2.19**	**0.029**
Offspring Success	26	**Intercept**	**1.48 ± 0.551**	**2.69**	**0.007**
		Year	1.6 ± 0.906	1.76	0.078
		DBB Similarity	-0.18 ± 0.826	-0.22	0.828
		DCB Similarity	0.9 ± 0.874	1.03	0.303
		DAB Similarity	0.27 ± 0.856	0.31	0.753
C. Genome-wide Similarity					
Copulation Success	89	**Intercept**	**-1.14 ± 0.132**	**-8.65**	**< 0.001**
		Year	**-0.66 ± 0.275**	**-2.42**	**0.016**
		Familiarity	0.36 ± 0.216	1.67	0.094
		Male Age	0.37 ± 0.235	1.59	0.111
		Similarity	0.19 ± 0.241	0.78	0.435
Breeding Success	53	**Intercept**	**-1.1 ± 0.232**	**-4.73**	**< 0.001**
		Year	**-1.78 ± 0.493**	**-3.62**	**< 0.001**
		Similarity	**0.89 ± 0.448**	**1.99**	**0.046**
Offspring Success	26	**Intercept**	**1.35 ± 0.561**	**2.41**	**0.016**
		Year	1.03 ± 0.965	1.07	0.284
		Similarity	0.72 ± 0.838	0.85	0.393

3 Bolded predictors show coefficients that are statistically different from 0 at the .05 alpha level
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Table 4 (on next page)

Effect of carrying specific MHCII alleles on male copulation, breeding and offspring success. > l,? G[l"

Only alleles that were present in more than one male were included. nt-->

Table 4. Effect of carrying specific MHCII alleles on male copulation, breeding and offspring success. Only alleles that were present in more than one male were included.

Response Variable	Locus	Allele*	n (0,1,2)	Slope ± SE	AIC_C	ΔAIC_C
Copulation Success	DBB	297	18/3/0	-0.952 ± 0.491	96.9	-
		Base	-	-	98.7	1.82
		287	17/4/0	-0.192 ± 0.213	99.9	3.00
		289	10/11/0	0.047 ± 0.196	100.6	3.76
		277	5/11/5	-0.004 ± 0.194	100.7	3.82
	DCB	**266**	**18/3/0**	**-1.201 ± 0.492**	**94.6**	**-**
		254	**16/5/0**	**-0.56 ± 0.247**	**95.3**	**0.73**
		220	18/3/0	0.532 ± 0.323	98.0	3.36
		226	18/3/0	0.311 ± 0.213	98.6	3.96
		Base	-	-	98.7	4.07
		250	18/3/0	0.381 ± 0.282	98.9	4.27
		260	19/2/0	-0.343 ± 0.288	99.2	4.61
		256	9/9/3	-0.228 ± 0.195	99.3	4.70
		252	18/3/0	0.3 ± 0.26	99.4	4.76
		228	19/2/0	0.364 ± 0.361	99.7	5.05
	DAB	Base	-	-	98.7	-
		289	15/6/0	0.335 ± 0.237	98.7	0.01
		297	14/4/3	0.089 ± 0.198	100.5	1.80
		285	14/7/0	-0.038 ± 0.204	100.6	1.97
		287	18/3/0	-0.362 ± 0.31	99.3	0.62
		291	13/7/1	-0.213 ± 0.196	99.5	0.80
		293	17/4/0	-0.181 ± 0.219	100.0	1.31
Breeding Success	DBB	**297**	**15/2/0**	**1.186 ± 0.427**	**67.0**	**-**
		Base	-	-	73.0	6.03
		289	7/10/0	-0.199 ± 0.325	74.7	7.65
		287	15/2/0	0.026 ± 0.327	75.0	8.02

Response Variable	Locus	Allele*	n (0,1,2)	Slope ± SE	AIC_C	ΔAIC_C
		277	3/10/4	-0.024 ± 0.308	75.0	8.02
	DCB	**266**	**15/2/0**	**1.186 ± 0.427**	**67.0**	**-**
		260	16/1/0	1.18 ± 0.511	69.1	2.12
		254	13/4/0	0.883 ± 0.41	70.3	3.26
		Base	-	-	73.0	6.03
		228	15/2/0	0.099 ± 0.507	75.0	7.99
		220	14/3/0	0.017 ± 0.634	75.0	8.03
		226	15/2/0	-0.936 ± 0.396	69.0	2.02
		252	14/3/0	-0.902 ± 0.419	70.1	3.11
		250	15/2/0	-0.619 ± 0.452	73.1	6.07
		256	6/9/2	-0.031 ± 0.319	75.0	8.02
	DAB	**289**	**11/6/0**	**-0.999 ± 0.394**	**68.2**	**-**
		291	11/6/0	0.56 ± 0.315	71.9	3.62
		293	14/3/0	0.511 ± 0.344	72.8	4.59
		Base	-	-	73.0	4.80
		285	12/5/0	0.192 ± 0.3	74.6	6.39
		287	14/3/0	-0.282 ± 0.481	74.7	6.46
		297	11/3/3	0.01 ± 0.319	75.0	6.80
Offspring Success	DBB	289	6/7/0	-0.863 ± 0.54	34.5	-
		297	11/2/0	-0.992 ± 0.617	34.6	0.03
		Base	-	-	35.2	0.67
		277	3/7/0	0.489 ± 0.576	36.4	1.92
		287	11/2/0	0.215 ± 0.512	37.0	2.49
	DCB	266	11/2/0	-0.992 ± 0.617	34.6	-
		Base	-	-	35.2	0.63
		228	11/2/0	0.825 ± 0.718	35.8	1.25
		250	11/2/0	0.824 ± 0.857	36.2	1.60
		226	11/2/0	0.744 ± 0.843	36.3	1.77

Response Variable	Locus	Allele*	n (0,1,2)	Slope ± SE	AIC_C	ΔAIC_C
		256	4/8/0	-0.413 ± 0.512	36.5	1.97
		252	10/3/0	0.317 ± 0.689	37.0	2.42
		254	11/2/0	0.208 ± 0.563	37.1	2.50
		220	11/2/0	0.085 ± 1.361	37.2	2.63
		260	12/1/0	-0.011 ± 0.582	37.2	2.63
	DAB	Base	-	-	35.2	-
		285	8/5/0	-0.618 ± 0.488	35.6	0.36
		293	10/3/0	0.295 ± 0.531	36.9	1.69
		287	11/2/0	0.33 ± 0.691	37.0	1.77
		297	9/3/0	0.226 ± 0.51	37.0	1.80
		289	8/5/0	-0.084 ± 0.587	37.2	1.98
		291	8/5/0	-0.056 ± 0.476	37.2	1.99

Models shown in bold show strong evidence that the respective allele influences the corresponding response variable due to the AIC_C values ranking highly (≥2 AIC_C) above the next best model and the intercept only model.

* All models are generalised linear models with response variables fitted as binomial trials (see Methods). All allele models include base parameters such as age and year (see Methods) plus a 1/0 binary predictor for presence/absence of the specified allele. Base models only include base parameters.

n represents the number of males carrying 0, 1 or 2 copies of the specified allele.