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Genetic relatedness in two-tiered plains zebra societies suggests that females choose to associate with kin

Wenfei Tong [a,*], **Beth Shapiro** [b] **and Daniel I. Rubenstein** [c,d]

[a] Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
[b] Department of Biology, The Pennsylvania State University, 326 Mueller Laboratory, University Park, PA 16802, USA
[c] Department of Ecology and Evolutionary Biology, 106A Guyot Hall, Princeton University, Princeton, NJ 08544-2016, USA
[d] Mpala Research Center, PO Box 155, Nanyuki, Kenya
[*] Corresponding author's e-mail address: wt249@cam.ac.uk

Abstract

How kinship structures alter inclusive fitness benefits or competition costs to members of a group can explain variation in animal societies. We present rare data combining behavioural associations and genetic relatedness to determine the influence of sex differences and kinship in structuring a two-tiered zebra society. We found a significantly positive relationship between the strength of behavioural association and relatedness. Female relatedness within herds was higher than chance, suggesting that female kin drive herd formation, and consistent with evidence that lactating females preferentially group into herds to dilute predation risk. In contrast, male relatedness across harems in a herd was no different from relatedness across herds, suggesting that although stallions benefit from associating to fend off bachelors, they do not preferentially form kin coalitions. Although both sexes disperse, we found that most harems contained adult relatives, implying limited female dispersal distances and inbreeding in this population, with potential conservation consequences.

Keywords

dispersal, fission–fusion society, modular society, microsatellites, inbreeding.

1. Introduction

Animal societies constitute one of the most varied and complex phenomena in biology, and explanations for their evolution, structural diversity and temporal stability hinge on the direct and indirect costs and benefits to individuals comprising a group. The spatio-temporal distribution of resources is

a classic predictor of both social and mating systems, as females tend to distribute themselves to minimise competition over food and water, while males distribute themselves to maximise access to mates (Rubenstein, 2009). In addition to bottom up pressures like food availability, top down pressures such as predation can also drive group formation (Hamilton, 1971; Rubenstein & Hack, 2004). If the individuals in a group are relatives, then inclusive fitness benefits could explain the maintenance of sociality (Hamilton, 1964), particularly when vigilance against predators or cuckolds is shared. However, living with relatives can come at the cost of increased kin competition if resources are limited, and can result in inbreeding depression unless at least one sex disperses (Clutton-Brock & Lukas, 2012).

In order to understand how these factors can influence social system variation, we focus on a relatively unusual mammalian system, the plains zebra (*Equus quagga*). Like a handful of social mammals, including some primates (Grueter et al., 2012), some whales (Whitehead et al., 1991; Baird, 2000) and prairie dogs (Hoogland, 1995), plains zebras live in two-tiered societies where stable core groups often form larger aggregations. Plains zebra core groups consist of groups of bachelor males or of harems comprising a stallion and several mares. While harems are closed-membership groups that are stable for years, they often join together with other harems or bachelor groups to form herds, which have fission–fusion dynamics in which both group size and composition vary across shorter timescales. Herds can range from 2 to 20 core groups, and often contain hundreds of individuals (Rubenstein & Hack, 2004).

A level of social organisation above the core group can serve a variety of adaptive functions otherwise unavailable to core group individuals. Banding together allows gelada baboon (*Theropithecus gelada*) and hamadrayas baboon (*Papio hamadryas*) core groups to lower predation risks (Dunbar, 1986; Schreier & Swedell, 2012). Similarly, lactating zebras benefit from associating with other herds of lactating females to dilute predation risk (Fischhoff et al., 2007; Rubenstein, 2010). Mate and resource defence are more effective in multi-harem clans of hamadryas baboons (Stammbach, 1978), and a comparative study shows that the 'bachelor threat hypothesis' appears to be the main selective explanation for multilevel societies in colobine primates (Grueter & van Schaik, 2010). The original inspiration for this hypothesis — plains zebra stallions — form coalitions that are more effective at driving off bachelors than are solitary stallions. Plains zebra bachelor groups are larger and persist for longer than those of other equids, such as wild horses, and

while a single horse stallion can drive away 2–3 bachelors, it takes more than a single zebra stallion to keep 9–10 coordinated bachelors at bay (Rubenstein, 1986; Rubenstein & Hack, 2004). These examples are consistent with the notion that higher levels of sociality form to solve problems that core structures cannot.

While the benefits of herd formation are apparent for plains zebras of both sexes, the costs are relatively low, as food intake rate does not increase significantly with herd size, an observation consistent with the formation of modular societies in colobine primates (Grueter & van Schaik, 2010). Competition between females in a harem is also low, as plains zebras inhabit mesic habitats where food and water are close together and food is moderately abundant and evenly distributed. In this system with harem polygyny, females also gain direct rewards from living in a group with a stallion to reduce sexual harassment by other males. Reduced harassment allows females to spend more time foraging, and increases their ability to find superior food (Rubenstein, 1986, 1994).

Behavioural evidence for direct benefits to both sexes at both levels of zebra society, coupled with low levels of competition for resources, imply that this two-tiered society could evolve independent of kin selection. Furthermore, observations from wild zebras show that unlike most group-living mammals, both sexes disperse in plains zebras societies (Klingel, 1967), suggesting that related individuals are unlikely to form a harem, or even a herd. This study is the first to attempt a fine-scaled genetic analysis of plains zebra societies using neutral microsatellite markers to estimate relatedness within and between harems and herds. We ask if kinship plays any role in structuring zebra societies at either level, and if so, which sex drives associations between and within harems. One hypothesis is that brother stallions band together to form kin-based alliances as a way to effectively defend their harems against bachelor males, and predicts that mean relatedness between stallions in a herd will exceed relatedness between stallions not observed in the same herd. A second hypothesis is that females actively drive associations with other harems to benefit from the protection of multiple stallions in reducing sexual harassment from bachelors, and that any additional competition costs associated with feeding in larger herds are amortised by preferentially sharing food with relatives. This would predict a higher female–female relatedness within than across temporary herd aggregations. A third, non-mutually exclusive hypothesis is that female kin band together as a by-product of their familiarity with the same areas and resources near their natal ranges. Like the

former hypothesis, this predicts higher female–female relatedness between harems in the same herd, but also within harems.

2. Material and methods

2.1. Location and field methods

The zebras in this study are from Ol Pejeta Conservancy, a semi-arid bushed grassland in the Laikipia highlands of central Kenya. We present data collected in 2004 and 2005 from a 100 km^2 section of the conservancy, known as Sweetwaters Game Reserve (0.043900, 36.932095). This Sweetwaters population consisted of approx. 350 zebras, and was surrounded by an electric fence until 2007, restricting movement to an area of 100 km^2 for 20 years.

The social dynamics of plains zebras on Ol Pejeta have been monitored since July 2003, by collecting association data at intervals of one day to one month. We drove set survey routes to find herds, and identified individual zebras by their unique stripe patterns. Using the 'gambit of the group' for defining association (Whitehead & Dufault, 1999; Franks et al., 2010), we assumed that all individuals less than 100 m apart were in the same herd, and considered all individuals in a herd to have associated with each other at that time. We defined clusters within each herd as distinct harems by watching which females continuously associated (within 20 m) with a single stallion during an observation session lasting from 30 min to multiple hours depending on the accessibility and observability of the herd. As temporally stable, closed membership groups, harems in our population are fundamentally different from herds, which are the result of temporary aggregations of harems. A total of 30 randomly selected harems from 2001 to 2015 in our study population remained stable for 0.5–7 years, with a median duration of 3.2 years without any change in harem membership (D.I.R., unpublished data).

For this study, we collected dung samples as a non-invasive source of DNA by waiting for individuals to defecate and noting their individual identity as well as their harem affiliations at the time of sampling in 2004 and 2005. As our primary aim was to opportunistically sample at least two individuals per social group, and zebra herds can fission and fuse rapidly, including when evading field biologists, we did not have the capacity to record the total number of harems in each herd sampled. Nor could we exhaustively sample or count all the harems in each herd. Rather, we focused on identifying each individual sampled, and relied on a database complied from

regular surveys to assign each to stable harems. If individuals from more than one harem were sampled at the same time within 100 m of each other, we assigned them to the same herd. Our genetic data represent a temporal snapshot of herd membership by harems recorded in a long-term database, with 1–5 harems successfully sampled for each of 35 herds. This yielded a total sample of 137 individuals from all 68 harems present in 2004–2005.

Dung samples were preserved using a modified version of the 2-step procedure described for primates (Nsubuga et al., 2004). From the surface of each dung ball 1–2 g was scraped to into 10-ml tubes and completely covered with 70–100% ethanol or RNAlater preservative solution (Ambion) within 1–5 min of defecation, as we only had RNAlater available later in the study. The ratio of solution to dung was 4:1; all samples were mixed by inversion, and stored at 4°C within 3–10 h of collection. After 24–36 h, the ethanol samples were centrifuged for 15 min at 3000 × g, the supernatant discarded, and the remaining pellet covered in silica. Silica beads had to be replaced 2–3 times over 24–36 h before pellets were dry enough to avoid changing the colour of the silica beads. All samples were stored then stored at −20°C for DNA extraction.

2.2. DNA extraction and microsatellite genotyping

The surface-scraped dung samples stored in 70–100% ethanol or RNAlater were extracted using a QIAamp DNA Stool Mini Kit® (Qiagen), according to the protocol outlined in Nsubuga et al. (2004). First, the entire dried pellet from ethanol and silica preservation was vortexed in 1.6 ml of ASL buffer and incubated at 25°C for 12–16 h. RNAlater samples were centrifuged for 15 min at 3000 × g, the supernatant removed and the resulting pellet resuspended in 1.6 ml of ASL buffer, then vortexed and incubated for 5 min at room temperature. All intermediate steps followed manufacturer's instructions. The final step, in which buffer AE elutes DNA, was modified to include an incubation step of 20 min at room temperature, followed by centrifugation for 2 min. All DNA extracts were stored at 4°C for 2–10 days in the field, and at −20°C upon returning to the laboratory.

In order to minimise the time necessary to develop microsatellite primers for zebras, we took advantage of 17 equine-specific microsatellite loci in the StockMarks for Horses Equine Genotyping Kit (Applied Biosystems). Each 17-plex polymerase chain reaction (PCR) contained a total volume of 15 μl (2.5 μl StockMarks PCR Buffer, 4 μl dNTP mix, 0.5 μl Ampli*Taq*

Gold DNA polymerase, 4 μl amplification primer mix, 1 μl deionised water, 1 μl bovine serum albumin (BSA) and 2 μl DNA template). PCRs were carried out in PTC-225 Peltier Thermal Cyclers (MJ Research) with the following conditions: 1 cycle of 10 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 60 s at 72°C, and a final cycle of 60 min at 72°C. Our PCR conditions for dung-extracted zebra DNA include three main modifications to the manufacturer's protocol: DNA template was increased from 1 μl to 2 μl to compensate for a relatively low concentration of zebra DNA from dung; deionised water was substituted with 1 μl BSA to bind residual proteins from the dung that could otherwise inhibit PCR; and the annealing temperature was reduced from 60 to 55°C, as cross-species PCRs often rely on a lower annealing temperature for less-specific primers to bind successfully (Smith et al., 2000; Galan et al., 2003).

All PCR samples were run on a 2% agarose gel to check for the presence of amplifications before being sent for automated capillary electrophoresis to separate PCR products (ABI PRISM® 3100). Alleles were sized relative to the internal size standard GeneScan 500LIZ and dye primer matrix standard DS-33 (Applied Biosystems). The 5′ end of each forward primer in the kit was labelled with one of four fluorescently coloured dyes to avoid confusing PCR products of similar length from different loci. We ran one negative control substituting DNA with water for every 30 PCR samples to check for contamination, and all negative controls did not yield amplification products.

Alleles at each microsatellite locus were scored using GeneMapper® Software v3.0 (Applied Biosystems), and independently rescored with a different genotyping program, STRand v.2.2.30 (Veterinary Genetics Laboratory, UC Davis). Both programs allow a combination of manual and automated scoring, whereby the user specifies the dye colour and allele size range for each locus (in bp). To minimise genotyping errors, 2–3 PCR replicates were performed for each DNA sample, and a genotype constructed by combining allele scores. An individual would be considered a heterozygote even if two different alleles appeared in separate replicate PCRs. In these cases, we assumed that the apparent homozygosity in individual PCRs was due to allelic dropout, a common problem encountered when amplifying degraded DNA, whereby only one allele at a heterozygous locus amplifies (Taberlet et al., 1999; Smith et al., 2000).

A total of 8 microsatellite primer pairs from the equine kit amplified successfully and were polymorphic in our plains zebra population; thus, our

analyses are restricted to these loci (VHL20, HTG4, HMS7, AHT5, HTG10, HTG7, HMS3, ASB17) (Tong, 2005). We confirmed that all these loci were segregating independently by randomising genotypes at locus pairs 10 000 times across all samples using FSTAT v2.9.3.2 (Goudet, 1995). Alleles at the same locus were randomised 10 000 times within and across populations to compute the probability that our data deviated from random mating (Hardy–Weinberg) expectations (Goudet, 1995). FSTAT calculates these probabilities for each locus and across all loci. GENEPOP v 3.4 (Raymond & Rousset, 1995a) was also used to test the hypothesis of heterozygote deficiency using the U-test (Rousset & Raymond, 1995b). P-values were estimated with 1000 dememorisations, 100 batches and 1000 iterations per population.

2.3. Relatedness analyses

Our final genetic sample contained 37 individuals from 10 herds, 7 of which were sampled for more than one individual in the same harem. We successfully sampled more than one zebra from a total of 9 harems, 3 of which were in the same herd. None of the individuals in our analyses were represented more than once. We restricted our analyses of genetic relatedness to 37 individuals because of the high failure rate of reliable microsatellite marker amplification from faecal DNA. Crucially, the number of loci can bias estimates of genetic relatedness (Altmann et al., 1996), and only a subset of individuals yielded DNA of sufficient quality and quantity to successfully amplify all 8 of the variable microsatellite loci in this study. We estimated Relatedness scores (R) for all pairwise combinations of the 37 zebras using RELATEDNESS v 5.0 (Queller & Goodnight, 1989). R can be underestimated if close relatives contribute to background allele frequencies (Queller & Goodnight, 1989; Altmann et al., 1996). To minimise this potential bias, average R excludes the herd, harem and Py (the group that an individual is being compared to) from calculations of the background allele frequencies. Jackknifing over loci, herds or core groups for each average relatedness value produced standard error estimates by dropping individual data points in turn, and recalculating R pseudovalues for each reduced data set. Relatedness scores were non-normally distributed because most pairs are non-relatives. We used the standard error associated with the highest number of pseudovalues, and computed and compared average R scores within a social and demographic category by computing, 'R-difference' values by subtracting one R from another. Significance levels represent the proportion

of jack-knifed R pseudovalues that coincide with the R-difference value calculated from our data.

2.4. Behavioural association analyses

An association index was calculated for each sampled dyad as half-weight indices $(2C/(A + B))$, where C is the number of times A and B were seen together within the same group, while $A + B$ is the total number of times A and B are seen with or without each other. For instance, if A and B are each seen 10 times and are seen together 10 times the association index is 1, while if each is seen 10 times and never seen together, the association index is 0. As harems are stable, long-term groups, association indices for harem members are typically 1, whereas association indices between members of different harems within temporary herds are between 0 and 1. Female plains zebras are in oestrous throughout the year in this location, so we did not expect seasonal differences in reproductive state when individuals were sampled to affect our conclusions about behavioural association or genetic relatedness. To see if genetic relatedness could predict the strength of social bonds, we performed a Spearman's correlation between pairwise genetic relatedness and the association index calculated for each genetically sampled dyad in 2004–2005. Statistical analyses were performed in JMP v 11.

2.5. Ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

3. Results

3.1. Relatedness within herds and harems

The mean relatedness of randomly selected dyads in our final sample was low ($R = -0.01 \pm 0.01$) (Table 1). Comparisons between different background estimates of R reveal that at the population level, average R across males, females and all male–female dyads did not differ significantly from the overall average R ($p > 0.05$) (Table 2). Jack-knifing results show that R between members of different harems or different herds was significantly lower than the overall background R ($p = 0.04$) (Table 2).

Table 1.

Mean relatedness scores (R) and standard errors (SE) for dyads of individuals in various demographic categories from the Sweetwaters plains zebra population ($N = 37$).

	Across 37 SW individuals				Across herds	Across harems	Within herds				Within core groups		
	All	M–M	F–M	F–F			All	M–M	F–M	F–F	All	F–M	F–F
Mean	−0.01	−0.02	−0.02	0.00	−0.02	−0.02	0.03	−0.09	0.04	0.09	0.18*	0.17*	0.30*
SD	0.22	0.21	0.23	0.24	0.22	0.22	0.25	0.23	0.27	0.20	0.23	0.27	0.12
SE	0.01	0.02	0.01	0.02	0.01	0.01	0.04	0.09	0.06	0.06	0.07	0.10	0.07
Upper 95% mean	0.00	0.01	0.01	0.04	0.00	0.00	0.11	0.12	0.16	0.23	0.34	0.41	0.60
Lower 95% mean	−0.03	−0.05	−0.04	−0.03	−0.03	−0.03	−0.05	−0.31	−0.08	−0.05	0.03	−0.08	0.01
N	666	171	342	153	627	655	39	7	22	10	9	7	2

All values in the table were calculated by averaging R values for all relevant dyads.

* Significant difference.

Table 2.
Comparisons of relatedness scores are calculated by subtracting one *R* value from another.

Comparison	R1	R2	R difference	p_{loci}	p_{herd}	p_{core}
Baseline relatedness within a population	M–M same population	Same population	−0.007	0.298	0.194	0.164
	F–M same population	Same population	0.007	0.298	0.195	0.140
	F–F same population	Same population	0.015	0.076	0.137	0.115
Across social groups	Different herds	Same population	−0.003	0.039*	0.357	0.421
	Different core groups	Same population	−0.003	0.001*	0.305	0.374
Between levels of social grouping	Same herd	Same population	0.083*	0.001*	0.000*	0.000*
	Same core group	Same population	0.1878	0.001*	0.000*	0.000*
	Same core group	Same herd	0.104*	0.024*	0.005*	0.002*
Within herds	M–M same herd	Same population	−0.117*	0.000*	0.025*	0.013*
	F–F same herd	F–F same population	0.110*	0.001*	0.000*	0.000*
	F–F same herd	Same herd	0.042	0.138	0.081	0.038*
	F–F same herd	M–M same herd	0.087*	0.008*	0.002*	0.001*
Within harems	F–M same harem	F–M same population	0.225*	0.000*	0.002*	0.001*
	F–M same harem	F–M same herd	0.124*	0.013*	0.024*	0.010*
	F–F same harem	F–M same harem	0.050	0.581	0.330	0.342
	F–F same harem	F–F same population	0.215*	0.001*	0.000*	0.000*
	F–F same harem	F–F same herd	0.104*	0.053*	0.004*	0.000*
	F–F same harem	Same harem	0.043	0.452	0.237	0.169

The first two columns specify the relationships for which *R* is being computed. The table is structured such that 5 estimates of baseline relatedness in the population are presented first, followed by comparisons across levels of social structure. The bottom two sections compare relationships between individuals from the same herd, and then the same harem. The, *R* difference column represents *R1-R2*. Positive values indicate that zebras in the first comparison (*R1*) are more closely related than zebras in the second comparison (*R2*). Negative values indicate that zebras in the first comparison (*R1*) are less closely related than zebras in the second comparison (*R2*).
* Significant difference.

Our final analyses focused on the associations within and between 9 of the 68 harems present in 2004 and 2005. In this genetic snapshot in time, we found that average relatedness was higher between herd members than between non-herd members (Table 1). Jack-knifing across loci, herds or harems showed that herd members were significantly more related than individuals randomly drawn from the sample genotype pool (R difference = 0.08; $p = 0.0005$). Harem members showed an even larger deviation from overall background relatedness (R difference = 0.19; $p = 0.001$), and relatedness within harems was also significantly larger than relatedness within herds (R difference = 0.10; $p = 0.02$) (Table 2).

3.2. Sex differences in relatedness

Female kinship accounts for this increased relatedness within social groups, as the average R of female harem members exceeds that of female herd members (R difference = 0.0421; $p = 0.0004$) (Table 2). This is in spite of the fact that only two female dyads were sampled from the same harem. Similarly, female herd members were significantly more related to each other than a randomly selected female dyad from the total sample (R difference = 0.11; $p = 0.001$). In spite of our limited sample size, female–male relatedness within harems was also significantly higher than female–male relatedness within herds (R difference = 0.12; $p = 0.01$). In contrast, males in the same herd were significantly less related to each other than they were to a randomly selected individual from the 37 sampled genotypes (R difference = -0.117; $p = 0.0002$).

3.3. Relatedness between individuals

Across both tiers of social organization, the strength of a social bond as measured by an association index was positively associated with pairwise genetic relatedness across all the zebras in our study, $r_s(664) = 0.36$, $p < 0.0002$ (Figure 1).

Although there was a significant relationship between association index and genetic relatedness in general, we found that pairwise relatedness within harems can vary widely from highly related dyads, to dyads that are no more related than chance (Figure A1 in the Appendix). We did not further subdivide these data by sex, due to the limited sample size. No consistent pattern across herds or harems appeared, as some harem members were highly related ($R = 0.48$–0.51), while other dyads had negative R values, and were no
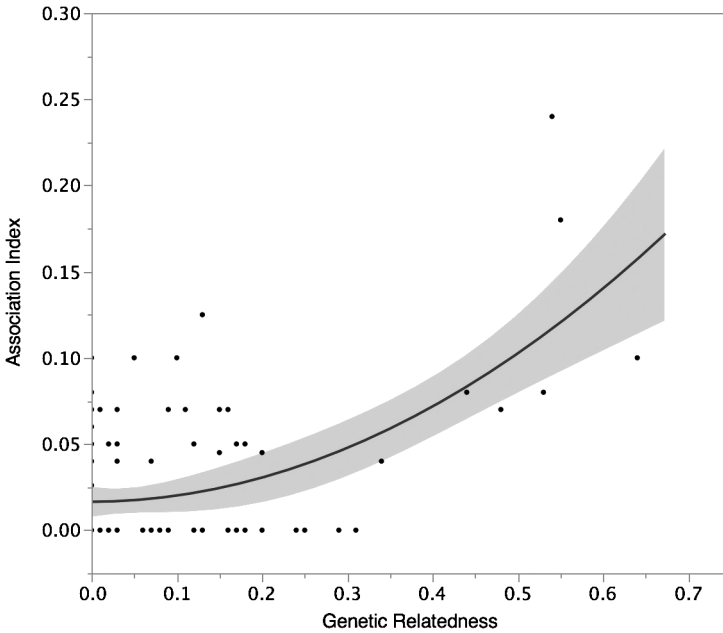
Figure 1. Graph of pairwise association index against pairwise genetic relatedness for all sampled zebra dyads in the Sweetwaters population, with quadratic regression line and shaded 95% confidence intervals. Dyads that are observed interacting as estimated by the association index are significantly more likely to be genetic relatives. An association index of zero indicates that a dyad of individuals was never seen together, while an index of one indicates that every time two individuals were seen, they were observed together in close proximity. Zero genetic relatedness is expected if a dyad of individuals does not share any alleles, while 0.5 is expected for first order relatives.

more related to each other than to the rest of the population. Similarly, herd members from different harems could be highly related ($R = 0.44, 0.58$) or unrelated ($R = 0$). For instance, the only juvenile in this sample of 37 individuals was a female, 02_149 that was from a different harem in the same herd as two mares. This juvenile female was closely related to one of the females ($R = 0.44$), but unrelated to the other ($R = -0.1$). However, both these adult females were as closely related as half-siblings ($R = 0.28$). This asymmetry in relatedness can be explained if, for instance, the two adult females were paternal half siblings, whereas the juvenile was related to only one of the females as a maternal half-sibling, but also through inbreeding between closely related parents.

4. Discussion

This study is a genetic snapshot in the social life of plains zebras that complements database information on long-term herd, harem and dyad associations. The genetic relationships among these associates begin to reveal the relationships between individual flexibility and decision-making on the time scale of days, with longer-term social bonds on the scale of months and years. As herds are temporary, fission–fusion groupings, our results represent a slice of time in the structuring of this two-tiered society. Our non-invasive sampling methods may have resulted in a small final sample size, but we are confident that our findings are representative of the total population, as we have no evidence the individuals that provided enough quality data for genetic analyses are a biased subset of our initial and reasonably comprehensive sample from all the harems present in 2004–2005. We found that on average, genetic relatedness predicts the strength of social bonds as measured by an association index calculated from behavioural observations (Figure 1). Without distinguishing between temporary herd and stable harem groupings, dyads with strong associations were significantly more likely to be related than dyads that were never seen together. These findings are consistent with the notion that on average, kin structure measured by genetic relatedness can echo behavioural estimates of social structure (Wolf et al., 2011), supporting the role of population structure in the evolution and maintenance of cooperation (Grafen, 2007). It would be particularly interesting for future studies involving more individuals to test for sex differences in kin association.

Classical behavioural ecology argues that social systems are largely dictated by females attempting to maximise resources, while males arrange themselves to maximise access to females (Rubenstein, 1986, 1994). Consistent with this hypothesis, estimated relatedness scores suggest that males in the same herd are less closely related than a random dyad of either sex chosen from the same population, so contrary to our first prediction, male kinship is not associated with herd formation, even though stallions gain from cooperating with kin to fend off bachelors (Rubenstein & Hack, 2004).

In contrast, high relatedness scores between females in the same herd relative to average female relatedness across the sampled genotypes is consistent with our second hypothesis that female kinship drives herd formation. At the proximate level of behavioural decisions, plains zebra herds tend to consist of harems with many females in the same reproductive state (Fischhoff et al., 2007). Females with young foals tend to associate with other females with

young foals, possibly to dilute the predation risk on their own offspring. Females also tend to dictate harem movements to food or water (Rubenstein, 1986), so a mutual assessment of reproductive state and assortment could occur when females from different harems choose to visit the same grazing areas or water sources. Females may gain direct benefits from herd formation by pooling their knowledge of resource locations, or diluting predation risk, while herding with relatives simultaneously reduces the small costs of reduced foraging efficiency and disease as group size increases. While these explanations are consistent with the inclusive fitness benefits of associating with female kin, they do not alter the substantial benefits to females of reduced sexual harassment when herding together enables stallions to drive bachelors away (Rubenstein & Hack, 2004). Additional behavioural studies are necessary to determine the mechanisms that tend to bring female kin from different harems together. Our observation of high genetic relatedness between females from different harems within herds could be a passive byproduct of limited dispersal and a tendency to congregate in important areas for food or water, or the result of kin recognition and communication between females that actively prefer to associate with relatives.

Our results demonstrate that the nature of natal dispersal in zebras and other equids may be more variable than previously thought. Equids are unusual among mammals in that both sexes typically disperse. Evidence from wild horses strongly suggests that female dispersal is largely driven by inbreeding avoidance, and not to reduce intrasexual competition (Monard & Duncan, 1996; Monard et al., 1996). Indeed, females sometimes remain spatially philopatric as long as inbreeding can be avoided (Linklater & Cameron, 2009). Our study sampled both sexes from 7 harems, 5 of which showed evidence of within-harem relatedness between females and the breeding stallion. This surprisingly high level of relatedness between adults of the opposite sex in a socially stable breeding group calls for a re-evaluation of plains zebra dispersal. Since Klingel (1967) has observed juvenile females switching harems several times between the time of natal dispersal and first reproduction, our genetic sample could represent a snapshot of how young females disperse. For instance, the single juvenile female sampled appears to share about half her genes with one of the adult females from a different harem in the same herd (Figure 1). However our study did not age females beyond two years, when dispersal may have yet to occur, particularly in a

crowded population like the one we sampled. A time-series of genetic samples would show how relatives move within the population over time to form the stable associations recorded in our behavioural database. Furthermore, larger studies in other populations will help to determine the generality of our genetic evidence suggesting limited dispersal.

The fact that females tend to be closely related to both their harem stallion and to other mares in the harem suggests that related individuals often end up dispersing to similar places, or that young females are quickly taken up by bachelor males who happen to be closely related because neither sex disperses very far. Even a random movement model would predict frequent re-acquaintances of related individuals if zebras typically disperse no more than one family group away (Rubenstein & Hack, unpublished observations, Ngorongoro Crater, Tanzania). Alternatively, the high relatedness between females in a harem could be explained by sisters dispersing together, or being more likely to join groups with familiar females that are likely to be relatives (Monard et al., 1996), but there is no demographic or behavioural evidence for this mechanism in plains zebras. Polygyny and long male tenures of at least 10 years suggest that only a small subset of males reproduces successfully, so zebra populations may be more locally inbred than aerial censuses suggest (Hack et al., 2002). High within-harem relatedness and consequent inbreeding could be adaptive, if the costs of inbreeding are relatively low, and females gain inclusive fitness benefits from mating with related males (Olson et al., 2012).

Alternatively, our finding of surprisingly high inbreeding opportunities between related stallions and adult mares in the same harem could be the product of land management practices altering the natural dispersal behaviour of plains zebras. In spite of morphological differences associated with geography, migratory populations of plains zebra show little genetic structuring across the species range (Lorenzen et al., 2008). In contrast, Laikipia (9666 km^2), the district where our study took place, comprises large commercial livestock ranches, small farms and game reserves, which provide supplemental water, allowing game populations to remain sedentary, and resulting in much higher levels of spatial genetic structuring, even between unfenced neighbouring ranches 25 km apart (Tong, 2005). Furthermore, GPS collar data show that even in these unfenced populations, individual home ranges are routinely similar to the total area enclosing the fenced population in our study (approx. 1000 km^2) (Tong et al., data not shown). As a result,

the unexpectedly high levels of genetic structuring and kinship within social groups we observe is not unusual for Laikipia, and more likely to be due to water provisioning than to fencing. Comparative studies from other managed populations would help explore the generality of our finding that the availability of water is a key driver of individual zebra movements, with effects on dispersal and the genetic identity of populations. In 1992, the Kenyan Wildlife Service issued permits for culling up to 15% of locally common wild herbivores per year. Plains zebras comprise almost half the total number of wild herbivores over 10 kg in weight, and are viewed as pests that compete with livestock for graze (Georgiadis et al., 2003). Yet in central Kenya, especially in Laikipia, plains zebra numbers have been increasing over the last decade, apart from temporary declines associated with a La Niña driven drought in 2000 (Georgiadis et al., 2007). Plains zebras in Kenya have had the behavioural flexibility to respond to both movement restrictions and culling, but these management practices could have consequences for the genetic and social structure of zebra societies.

Acknowledgements

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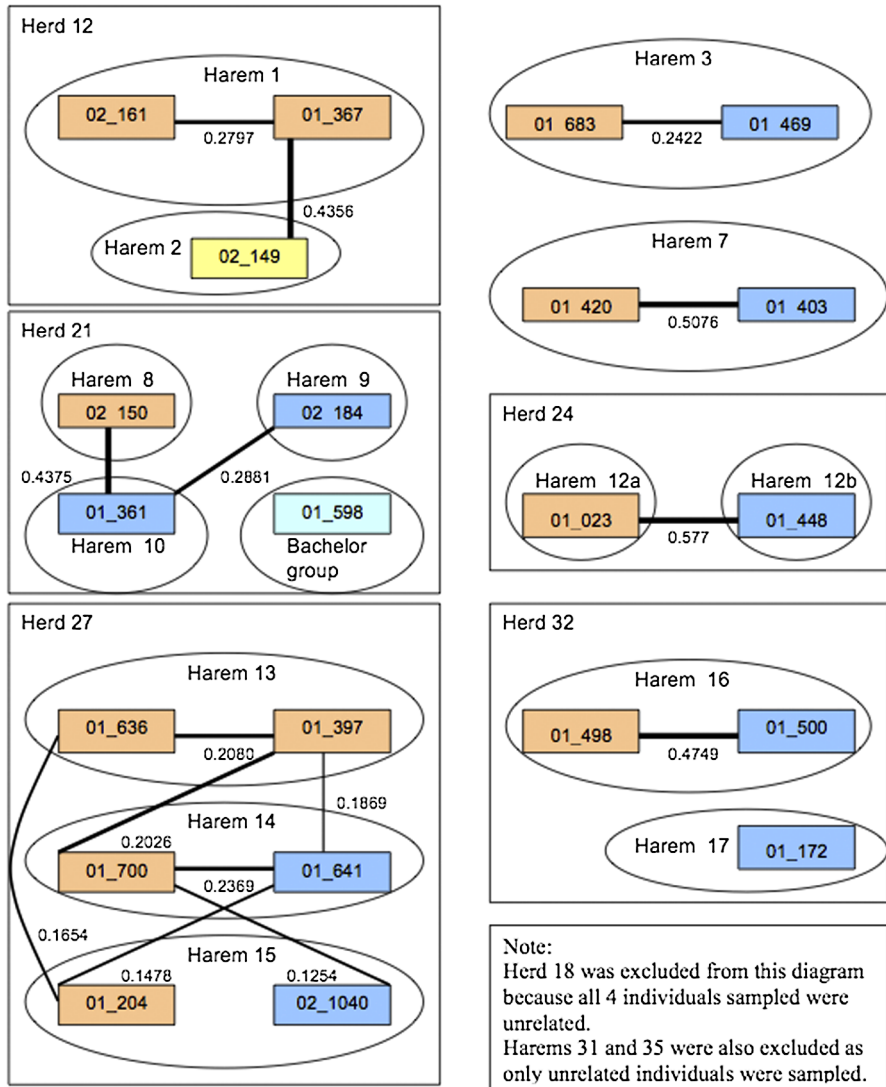
Appendix

Figure A1. Pairwise relatedness between members of herds and harems in the Sweetwaters Game Reserve. Females are peach, stallions blue, bachelors pale blue and the only female juvenile is yellow. Harems are enclosed by ovals, while herds are enclosed by rectangles. Only relatedness scores > 0.1 are represented, and line thickness joining individuals indicates the strength of relatedness. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.