CRYPTIC CONSEQUENCES OF PARASITISM

by

KATHERINE WORSLEY-TONKS

(Under the Direction of Vanessa Ezenwa)

ABSTRACT

Parasites can have a range of negative consequences on individuals and populations. We explored mechanisms by which parasitic worms affect the fitness of Grant's gazelles (*Nanger granti*). To do this, we first assessed how parasites influenced time allocation to competing behaviors in female gazelles. Second, we investigated when parasites are more likely to impose costs on individuals with lower levels of genetic diversity, or heterozygosity. We showed that parasites can affect the time female gazelles allocate to foraging versus vigilance, which may have important implications for individual predation risk. We also showed that less genetically diverse animals may suffer from higher parasite burdens but only under certain environmental conditions and for certain individuals. Overall, our work demonstrates that parasites can exert effects on hosts via cryptic mechanisms that are manifested through host behavior or that occur only during specific times or for specific individuals.

INDEX WORDS: Animal behavior, Anthelmintic treatment, Cryptic, Fitness Consequence, Grant's gazelle, Heterozygosity, Microsatellite, Nematode

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KATHERINE WORSLEY-TONKS

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KATHERINE WORSLEY-TONKS

Major Professor:	Vanessa Ezenwa
Committee:	Stacey Lance
	Andrew Park

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Parasites exert strong selection pressures on their hosts in multiple different ways. Parasites can directly affect host survival, but in many cases the effects of parasites on host fitness occur in more subtle and complex ways. For instance, macroprasites (e.g. helminths and arthropods) often impose costs on hosts that manifest as changes in physiology, morphology or behavior which can then impact host survival or reproduction (Zuk et al. 1998; Irvine et al. 2006; Schmid-Hempel 2011), or the lethal effects of parasites may occur only among certain subsets of individuals or at certain times. For example, the effects of parasites on condition and performance may alter an individual's behavioral response, which might reduce its ability to raise offspring. In Cape ground squirrels (*Xerus inauris*) for instance, Hillegass et al. (2010) found that females relieved of ecto- and endoparasites invested more energy into raising offspring and less into improving their own condition, suggesting that parasites were directly responsible for reducing host reproductive success. However in some instances, parasites can have negative effects on some host individuals but not others, leading to differences in fitness. Males, for example, are often more susceptible to parasite infections than females (Kamis et al. 1992; Grear et al. 2009), and in Soay sheep (*Ovis aries*) experimental removal of gastrointestinal parasites actually reversed male-biased mortality (Gulland et al. 1993). Similarly, several studies have illustrated that parasites play a critical role in maintaining genetic diversity by selecting against inbred individuals (Coltman et al. 1999; Acevedo-Whitehouse et al. 2003; Hawley et al. 2005; Rijks et al. 2008; Ruiz-Lopez et al. 2012). In addition, parasites may act against certain

individuals in even more cryptic ways by participating in other biological interactions. In snowshoe hares (*Lepus americanus*) for instance, survival in response to predation was 2.4 times higher for individuals treated with an anthelmintic drug than controls (Murray et al. 1997). While in other instances, the fitness consequences of infection may occur at specific times such as when environmental conditions are unfavorable. For example, in red grouse (*Lagopus lagopus scoticus*), Hudson et al. (1992) found that individuals treated with an anthelmintic were more likely to survive harsh winters than control individuals, suggesting that parasites were directly responsible for winter mortalities.

One other important consequence of infection is that parasites might alter how much time animals invest in essential behaviors. Since, time is limited, and extra time invested in one behavior will inevitably take time away from other behaviors, animals have to optimize their time effectively. Effective time optimization generally depends on the physiological status and ecological requirements of individuals (Bachman et al. 1993; Illius et al. 2002; Edwards et al. 2013). Several intrinsic and extrinsic factors can influence how much time animals devote to different behaviors. For example, life-history traits such as sex (Key & Ross 1999; Prates & Bicca-Marques 2008), age (Ruckstuhl et al. 2003; Gelin et al. 2013), reproductive state (Hamel & Cote 2008), and social rank (Main 2008; Pelletier & Festa-Bianchet 2004) may all play a role in influencing how much time animals invest in competing behaviors. Likewise, social drivers such as group size may predict how much time animals will invest between foraging and vigilance behaviors (Blumstein et al. 2003; Childress & Lung 2003; Hopewell et al. 2005; Watson et al. 2007; Lashley et al. 2014). Parasites can also influence how much time animals invest on specific behaviors. In scincid lizards (*Egernia stokesii*), individuals relieved of their nematode infections invested more time in basking behaviors than controls (Fenner & Bull

2008). Similarly, insect harassment predicted how much time reindeer (*Rangifer tarandus*) invested in grooming over foraging behaviors (Witter et al. 2012), suggesting that parasites can act as important drivers of time allocation. Hence, parasites might indirectly affect host fitness by altering time investment to competing behaviors.

However, not all individuals within a population are likely to suffer the same degree of parasitism. Indeed, parasite distributions are notoriously heterogeneous across hosts (Wilson et al. 2002). Similarly, the effects of parasite infections on hosts might vary, with certain individuals experiencing greater negative impacts. For instance, more inbred individuals are often more likely to suffer from higher parasite burdens than outbred individuals (Coltman et al. 1999; Acevedo-Whitehouse et al. 2003; Hawley et al. 2005; Rijks et al. 2008; Ruiz-Lopez et al. 2012). One common approach of exploring the effects of inbreeding on parasite infection is to examine associations between infection and individual genetic diversity, or heterozygosity, estimated from molecular markers (Coltman et al. 1999; Acevedo-Whitehouse et al. 2006; Charpentier et al. 2008; Ruiz-Lopez et al. 2012; Shaner et al. 2013). However, heterozygosity-fitness correlations (HFCs) more generally, are highly inconsistent across studies (e.g. Acevedo-Whitehouse et al. 2006; Rijks et al. 2008; Chapman et al. 2009; Fox & Reed 2011; Harrison et al. 2011). Recent studies suggest that possible reasons for this inconsistency may be differences in the fitness-related traits examined; in individual characteristics (e.g. Pujolar et al. 2006; Szulkin et al. 2007; Shaner et al. 2013), and in the environmental condition that individuals experience (Lense et al. 2000; Lesbarreres et al. 2005; Pujolar et al. 2006; Szulkin et al. 2007; Harrison et al. 2011; Annavi et al. 2014).

This thesis explored the complex ways by which parasitic worms negatively affect host fitness using two approaches. The first aim was to investigate how parasites affect individual

hosts by altering time investment to competing behaviors, and the second was to examine when parasites were more likely to affect less genetically diverse individuals. These questions were explored in a Grant's gazelle (*Nanger granti*) population in Kenya. The Grant's gazelle is a common and widely distributed East African antelope species, and the often high rates of gastrointestinal and pulmonary nematode infections makes this species an ideal model organism to explore the potential fitness consequences of parasitism (Ezenwa 2003, 2004; Ezenwa et al. 2012).

Chapter 2 explores whether parasites influence time allocation to competing behaviors in female gazelles. Specifically, the goal was to examine how removal of gastrointestinal and pulmonary worms influenced time investment to essential behaviors including foraging, vigilance, resting, and moving. The anthelmintic treatment effectively removed worm infections, during which time treated females invested more time foraging at the expense of vigilance. This work demonstrates that parasites can impose trade-offs on host behavior that are equivalent in magnitude to commonly studied social drivers of behavioral time allocation, like group size. Chapter 3 explored the relationship between individual heterozygosity and parasite infection, and examined whether these associations varied with host characteristics (e.g. age) and environmental conditions (e.g. seasonality). Using two different approaches, cross-sectional and longitudinal sampling, the study revealed that associations between heterozygosity and parasite infection vary with context, and that the relevant context differs for different parasite taxa. This work demonstrates that less genetically diverse animals may suffer from higher parasite burdens but only under certain conditions and at certain times.

REFERENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D., & Amos, W. (2003). Inbreeding: disease susceptibility in California sea lions. *Nature*, *422*(6927), 35-35.

Acevedo-Whitehouse, K. A. R. I. N. A., Spraker, T. R., Lyons, E., Melin, S. R., Gulland, F., Delong, R. L., & Amos, W. (2006). Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups.*Molecular Ecology*, *15*(7), 1973-1982.

Annavi, G., Newman, C., Buesching, C. D., Macdonald, D. W., Burke, T., & Dugdale, H. L. (2014). Heterozygosity–fitness correlations in a wild mammal population: accounting for parental and environmental effects. *Ecology and evolution*, *4*(12), 2594-2609.

Bachman, G. C. (1993). The Effect of Body Condition on the Trade-Off Between Vigilance and Foraging in Belding Ground-Squirrels. *Animal Behaviour, 46*(2), 233-244. doi: 10.1006/anbe.1993.1185

Blumstein, D. T., Daniel, J. C., & Sims, R. A. (2003). Group size but not distance to cover influences agile wallaby (Macropus agilis) time allocation. *Journal of Mammalogy, 84*(1), 197-204. doi: 10.1644/1545-1542(2003)084<0197:gsbndt>2.0.co;2

Chapman, J. R., Nakagawa, S., Coltman, D. W., Slate, J., & Sheldon, B. C. (2009). A quantitative review of heterozygosity–fitness correlations in animal populations. *Molecular Ecology*, *18*(13), 2746-2765.

Charpentier, M. J., Williams, C. V., & Drea, C. M. (2008). Inbreeding depression in ring-tailed lemurs (Lemur catta): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics*, *9*(6), 1605-1615.

Childress, M. J., & Lung, M. A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour, 66*, 389-398. doi: 10.1006/anbe.2003.2217

Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, 1259-1267.

Edwards, A. M., Best, E. C., Blomberg, S. P., & Goldizen, A. W. (2013). Individual traits influence vigilance in wild female eastern grey kangaroos. *Australian Journal of Zoology, 61*(4), 332. doi: 10.1071/zo13025

Ezenwa, V. O. (2003). Habitat overlap and gastrointestinal parasitism in sympatric African bovids. *Parasitology, 126*(4), 379-388. doi: 10.1017/s0031182002002913

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology, 15*(3), 446-454. doi: 10.1093/beheco/arh028

Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology, 26*(1), 123-133. doi: 10.1111/j.1365-2435.2011.01919.x

Fenner, A. L., & Bull, C. M. (2008). The impact of nematode parasites on the behaviour of an Australian lizard, the gidgee skink Egernia stokesii. *Ecological Research, 23*(5), 897-903. doi: 10.1007/s11284-007-0453-1

Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution, 65*(1), 246-258.

Gélin, U., Wilson, M. E., Coulson, G. M., & Festa-Bianchet, M. (2013). Offspring sex, current and previous reproduction affect feeding behaviour in wild eastern grey kangaroos. *Animal Behaviour, 86*(5), 885-891. doi: 10.1016/j.anbehav.2013.08.016

Grear, D. A., Perkins, S. E., & Hudson, P. J. (2009). Does elevated testosterone result in increased exposure and transmission of parasites?.*Ecology letters, 12*(6), 528-537.

Gulland, F. M. D., Albon, S. D., Pemberton, J. M., Moorcroft, P. R., & Clutton-Brock, T. H. (1993). Parasite-associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *254*(1339), 7-13.

Hamel, S., & Côté, S. D. (2008). Trade-offs in activity budget in an alpine ungulate: contrasting lactating and nonlactating females. *Animal Behaviour, 75*(1), 217-227. doi: 10.1016/j.anbehav.2007.04.028

Harrison, X. A., Bearhop, S., Inger, R., Colhoun, K., Gudmundsson, G. A., Hodgson, D., & Tregenza, T. O. M. (2011). Heterozygosity–fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects. *Molecular ecology*, *20*(22), 4786-4795.

Hawley, D. M., Sydenstricker, K. V., Kollias, G. V., & Dhondt, A. A. (2005). Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. *Biology Letters*, *1*(3), 326-329.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology*, arq041.

Hopewell, L., Rossiter, R., Blower, E., Leaver, L., & Goto, K. (2005). Grazing and vigilance by Soay sheep on Lundy island: Influence of group size, terrain and the distribution of vegetation. *Behavioural Processes, 70*(2), 186-193. doi: 10.1016/j.beproc.2005.04.009

Hudson, P. J., Newborn, D., & Dobson, A. P. (1992). Regulation and stability of a free-living host-parasite system: Trichostrongylus tenuis in red grouse. I. Monitoring and parasite reduction experiments. *Journal of animal ecology*, 477-486.

Illius, A. W., Duncan, P., Richard, C., & Mesochina, P. (2002). Mechanisms of functional response and resource exploitation in browsing roe deer. *Journal of Animal Ecology, 71*(5), 723-734. doi: 10.1046/j.1365-2656.2002.00643.x

Irvine, R. J., Corbishley, H., Pilkington, J. G., & Albon, S. D. (2006). Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (Cervus elaphus). *Parasitology*, *133*(04), 465-475.

Kamis, A. B., Ahmad, R. A., & Badrul-Munir, M. Z. (1992). Worm burden and leukocyte response inAngiostrongylus malaysiensis-infected rats: the influence of testosterone. *Parasitology research*, *78*(5), 388-391.

Key, C., & Ross, C. (1999). Sex differences in energy expenditure in non-human primates. *Proceedings of the Royal Society B-Biological Sciences, 266*(1437), 2479-2485.

Lashley, M. A., Chitwood, M. C., Biggerstaff, M. T., Morina, D. L., Moorman, C. E., & DePerno, C. S. (2014). White-tailed deer vigilance: the influence of social and environmental factors. *PLoS One, 9*(3), e90652. doi: 10.1371/journal.pone.0090652

Lesbarreres, D., Primmer, C. R., Laurila, A., & Merilae, J. (2005). Environmental and population dependency of genetic variability-fitness correlations in Rana temporaria. *Molecular Ecology*, *14*(1), 311-323.

Lens, L., Van Dongen, S., Galbusera, P., Schenck, T., Matthysen, E., & Van de Casteele, T. (2000). Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. *Journal of Evolutionary Biology, 13*(6), 889-896.

Main, M. B. (2008). Reconciling competing ecological explanations for sexual segregation in ungulates. *Ecology*, *89*(3), 693-704. doi: 10.1890/07-0645.1

Murray, D. L., Cary, J. R., & Keith, L. B. (1997). Interactive effects of sublethal nematodes and nutritional status on snowshoe hare vulnerability to predation.*Journal of Animal Ecology*, 250-264.

Pelletier, F., & Festa-Bianchet, M. (2004). Effects of body mass, age, dominance and parasite load on foraging time of bighorn rams, Ovis canadensis. *Behavioral Ecology and Sociobiology, 56*(6), 546-551. doi: 10.1007/s00265-004-0820-7

Prates, H. M.,& Bicca-Marques, J. C. (2008). Age-sex analysis of activity budget, diet, and positional behavior in Alouatta caraya in an orchard forest. *International Journal of Primatology, 29*(3), 703-715. doi: 10.1007/s10764-008-9257-6

Pujolar, J. M., Maes, G. E., Vancoillie, C., & Volckaert, F. A. M. (2006). Environmental stress and life-stage dependence on the detection of heterozygosity-fitness correlations in the European eel, Anguilla anguilla.*Genome*, *49*(11), 1428-1437.

Rijks, J. M., Hoffman, J. I., Kuiken, T., Osterhaus, A. D. M. E., & Amos, W. (2008). Heterozygosity and lungworm burden in harbour seals (Phoca vitulina).*Heredity*, *100*(6), 587-593.

Ruckstuhl, K. E., Festa-Bianchet, M., & Jorgenson, J. T. (2003). Bite rates in Rocky Mountain bighorn sheep (Ovis canadensis): effects of season, age, sex and reproductive status. *Behavioral Ecology and Sociobiology, 54*(2), 167-173. doi: 10.1007/s00265-003-0615-2

Ruiz-López, M. J., Gañan, N., Godoy, J. A., Del Olmo, A., Garde, J., Espeso, G. & Gomendio, M. (2012). Heterozygosity-Fitness Correlations and Inbreeding Depression in Two Critically Endangered Mammals. *Conservation Biology, 26*(6), 1121-1129.

Schmid-Hempel, P. (2011). *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics* (p. 496). New York: Oxford University Press.

Shaner, P. J. L., Chen, Y. R., Lin, J. W., Kolbe, J. J., & Lin, S. M. (2013). Sex-specific correlations of individual heterozygosity, parasite load, and scalation asymmetry in a sexually dichromatic lizard. *PloS one*, *8*(2), e56720.

Szulkin, M., Garant, D., McCleery, R. H., & Sheldon, B. C. (2007). Inbreeding depression along a life-history continuum in the great tit. *Journal of evolutionary biology*, *20*(4), 1531-1543.

Watson, M., Aebischer, N. J., & Cresswell, W. (2007). Vigilance and fitness in grey partridges Perdix perdix: the effects of group size and foraging-vigilance trade-offs on predation mortality. *J Anim Ecol, 76*(2), 211-221. doi: 10.1111/j.1365-2656.2006.01194.x

Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Randolph, S. E. & Skorping, A. (2002). Heterogeneities in macroparasite infections: patterns and processes. *The ecology of wildlife diseases*, 6-44.

Witter, L. A., Johnson, C. J., Croft, B., Gunn, A., & Gillingham, M. P. (2012). Behavioural trade-offs in response to external stimuli: time allocation of an Arctic ungulate during varying intensities of harassment by parasitic flies. *J Anim Ecol, 81*(1), 284-295. doi: 10.1111/j.1365-2656.2011.01905.x

Zuk, M., Kim, T., Robinson, S. I., & Johnsen, T. S. (1998). Parasites influence social rank and morphology, but not mate choice, in female red jungle fowl, Gallus gallus. *Animal Behaviour*, *56*(2), 493-499.

CHAPTER 2

ANTHELMINTIC TREATMENT AFFECTS BEHAVIORAL TIME ALLOCATION IN A FREE-RANGING UNGULATE[1]

[1] Worsley-Tonks, K.E.L. and Ezenwa, V.O. Submitted to *Animal Behaviour*

ABSTRACT

Social, ecological and environmental factors all influence how much time animals allocate to different behaviours. Here, we investigated whether parasites affect behavioural time allocation in a free-ranging ungulate that must apportion time to multiple, competing activities crucial for maintenance and survival. We examined how experimental removal of gastrointestinal and pulmonary nematodes influenced relative amounts of time female Grant's gazelles (*Nanger granti*) allocated to core behaviours including foraging, vigilance, moving, and resting. The anthelmintic treatment effectively reduced female parasite load for ~120 days, and during this period females relieved of their parasitic nematodes adjusted their daily time budgets. At the group level, parasite removal resulted in an increase in foraging time and a decrease in vigilance. This effect was also apparent at the individual level where treated females allocated more time to foraging at the expense of vigilance. In addition to treatment, group size was a significant predictor of the relative time spent in foraging versus vigilance, where females in larger groups allocated more time to foraging at the expense of vigilance. Our results suggest that parasites can induce changes in host behaviour that are of similar magnitude to some of the most commonly studied social drivers of behavioural time allocation.

INTRODUCTION

The amount of time animals can allocate to essential daily activities is limited. Time allocated to one behaviour takes time away from mutually exclusive behaviours that are equally important for survival and reproduction (Sterns et al. 1992; Sibbald & Hooper 2004; Rauter & Moore 2004; Dunbar et al. 2009). In most cases, animal time budgets revolve around resource

acquisition (Dunbar et al. 2009). However, time and energy must also be invested in anti-predator and reproductive behaviours, and in some cases in forming social relationships. Animals therefore face constraints on how much time they can devote to competing activities, and must frequently substitute one behaviour for another. How individuals optimise these time allocation decisions often depends on their physiological status and ecological requirements (Bachman et al. 1993; Illius et al. 2002; Edwards et al. 2013).

A variety of intrinsic and extrinsic factors determine the relative amounts of time individuals allocate to different behaviours. For instance, during lactation, female Mountain goats (*Oreamnos americanus*) allocate more time to foraging at the expense of time spent resting to meet their increased energetic demands (Hamel & Cote 2008). Other life-history and social traits such as age (Ruckstuhl et al. 2003; Gelinet al. 2013), sex (Key & Ross1999; Prates & Bicca-Marques 2008), and group size (Creel et al. 2014; Lashley et al. 2014; Michelena et al. 2011) also affect time allocation to competing behaviours. For example, both group size and adult sex ratio influence time allocation to major activities in alpine ibex (*Carpa ibex*, Tettamanti & Viblanc 2014). More generally, group size is recognised as being a major determinant of how much time animals invest in foraging and vigilance (Creel et al. 2014; Fuller et al. 2013; Halupka & Osinska-Dzienniak 2013). When group sizes are large individuals often invest more time foraging and less time being vigilant, but the strength of this effect depends on other factors such as predator abundance (e.g. Cresswell 1994), group composition (e.g. Tettamanti & Viblanc 2014), and individual status (e.g. Powolny et al. 2014). Abiotic factors also play a role; when food is abundant individuals often increase time invested in resource acquisition and decrease time spent on vigilance (Ruckstuhl et al. 2003) and other activities such as social behaviour (Alberts et al. 2005). In contrast, when resources are patchily distributed, individuals often invest

more time in movement behaviours and less on feeding and vigilance (Kotler et al. 1994; Tadesse & Kotler 2014), illustrating the complex ways in which social, ecological, and environmental factors shape animal time budgets.

Parasites can have profound effects on animal behaviour, including changing the amounts of time individuals invest in specific activities. In Australian scincid lizards (*Egernia stokesii*), individuals relieved of nematode infections spend almost five-fold more time basking compared with controls (Fenner & Bull 2008). Similarly, dairy cattle relieved of nematode infections graze ~50 minutes longer per day than do controls (Forbes et al. 2004, 2007). Feeding depression, where infected individuals voluntarily reduce forage intake, is actually a common by-product of gastrointestinal nematode infection in domestic ruminants (Coop & Holmes 1996). These types of direct effects of parasites on host behaviour may also influence how much time animals allocate to competing activities. For instance, a recent study on reindeer (*Rangifer tarandus*) found that insect harassment resulted in individuals investing more time grooming at the expense of foraging (Witter et al.2012), suggesting that parasites induced a re-allocation of time between resource acquisition and parasite defence.

Here, we investigated whether parasitic nematodes can influence behavioural time allocation in Grant's gazelles (*Nanger granti*). Grant's gazelles experience high rates of gastrointestinal and pulmonary nematode infection (Ezenwa 2004, Ezenwa et al. 2012); and given the strong direct effects that nematodes can have on livestock feeding behaviour (Arneberg et al. 1996; Fox 1997; Forbes et al. 2000), our goal was to establish whether these parasites influence time allocation in a species that must balance multiple, competing activities crucial for maintenance and survival (e.g. foraging, anti-predator behaviour, movement).We manipulated female gazelle parasite loads using an anthelmintic drug to test, first, the direct effects of parasite

removal on major components of the daily activity budget including foraging, vigilance, moving, resting and other behaviours; and second, the potential effect of parasite removal on individual time allocation decisions. We predicted that parasite treatment would counteract nematode-induced feeding depression in gazelles resulting in an increase in foraging time. We also expected that an increase in time spent foraging would be accompanied by reductions in one or more other activities reflecting a re-allocation of time in response to parasite treatment.

METHODS

Study Animals

We studied the behaviour of female Grant's gazelle at the Mpala Research Centre (MRC), Kenya ($0^{o}17'$N, $37^{o}52'$ E) from 20 June 2011 to 30 April 2012. Gazelles were captured and ear-tagged over a five day period in June 2011 as part of a long-term study of parasitism and host behaviour (Ezenwa et al. 2012). Animals were located by helicopter and captured using a hand-held net gun fired from the aircraft. A single observer (VOE) collected information on individual morphometrics, including horn length (distance between the base and tip of horn on both the right and left sides) to facilitate age estimation. For females, age was estimated from an equation relating horn length to tooth wear developed for a subset of nine individuals from the same population (Ezenwa, unpublished data; see also Spinage1976). To experimentally assess the effects of nematodes on host behaviour, all captured females were randomly assigned to an anthelmintic treatment group (treated vs. control) based on the temporal sequence of capture. Prior to group assignment, faecal samples were collected from all individuals for parasitological analysis. Treated individuals received a subcutaneous injection of moxidectin (1ml/20kg of Cydectin Long Acting Injection for Sheep, Virbac Animal Health). This drug provides protection against a broad range of nematodes for ~120 days in sheep (Papadopoulos et al. 2009).

Average handling time per animal was 17 minutes and all possible precautions were taken to minimize stress. Throughout the process, animals were monitored by a wildlife veterinarian. Because no drugs were used to subdue captured females, individuals resumed normal behaviour within minutes of release. Captures were performed under the authority of the Kenya Wildlife Service. Animal protocols were approved by the University of Georgia IACUC (#A2010 10-188) and conformed to the guidelines for the treatment and use of animals in behavioural research (ASAB/ABS 2011).

Behavioural Observations

We monitored the behaviour of 9 treated and 9 control females for approximately nine months, from 26 July-30 November 2011 and 5 January-30 April 2012 using focal animal sampling (Altmann 1974). Behavioural observations were taken from a vehicle or on foot from a distance of 100- 200 meters using binoculars and a hand-held digital voice recorder. To begin a focal observation we located a group of females and then randomly selected one individual that was in clear view. We paused the recording if the focal individual went out of sight, and if the individual was out of sight for more than ~10 minutes the observation was terminated. Observations shorter than 15 minutes were excluded from the dataset. A single observer (KWT) performed 454 focal observations ranging in duration from 15-26 minutes (average: 20.2 minutes). The average number of observations per female was 25.2 (range: 7-35).

We classified behaviours into five categories: (1) foraging, (2) vigilance, (3) resting, (4) moving and (5) other activities. Foraging involved feeding at any height (e.g. grazing or browsing) or actively searching for food. Vigilance was defined as head-up awareness where an animal raised its head above shoulder height and was actively looking around with ears cocked (Geist 1971; Fird 1977; Brivio et al. 2014). To capture aspects of vigilance and foraging that are

mutually exclusive, we coded a behaviour as vigilance, not foraging, if an individual interrupted a foraging bout to raise its head and look around, even if it was still handling food (e.g. chewing). Resting was considered as periods when individuals were either standing or lying while idle. Resting periods often corresponded to rumination bouts, but we did not distinguish between resting and rumination. If an individual became vigilant while resting, the period of time during which the animal's head was raised with ears cocked was coded as vigilance not resting. Moving included directional movement either walking or running; other activities included agonistic, reproductive, and maintenance behaviours such as grooming and defecating.

To account for potential effects of time of day on gazelle activity, we distributed focal observations across four time periods: early morning (0600-0859), late morning (0900-1159), early afternoon (1200-1459), and late afternoon (1500-1759). All behaviour observations were terminated after 1800 hours because African antelope are typically inactive after dark (Walther 1969, 1973). For each observation we recorded the date, start time, weather (clear, overcast or rainy), wind conditions (low or high), and the size and type of group containing the focal female. Group type was classified according to sex and age composition as: females with juveniles (nursery groups), nursery groups in the company of a territorial male, or nursery groups with bachelor males.

Parasitological Analyses

Faecal samples collected prior to the anthelmintic treatment (i.e. during capture in June 2011) and throughout the behavioural observation period (July 2011-April 2012) were used to monitor the effects of treatment on parasite infection. At capture, we collected faecal samples directly from the rectum of all females. In all other instances, we collected samples opportunistically from known individuals within 10 minutes of observing a defecation event.

Faecal samples were collected between ~0630 and 1830 hours, 2-3 times per month for each female. Animal ID, time of day, and location were recorded for all samples collected. After collection, the samples were kept on ice in the field until being transported to the lab for processing.

We focused our parasitological analyses on gastrointestinal and pulmonary nematodes which occur at high prevalence in Grant's gazelle (Ezenwa 2003, 2004; Ezenwa et al. 2012). We quantified faecal egg output of strongyle nematodes using a modification of the McMaster egg counting technique (Ezenwa 2003). First-stage lungworm larvae in faeces were measured using a beaker-modified Baermann method (Forrester & Lankester 1997; Ezenwa et al. 2012). All samples were processed on the day of collection. A total of 270 faecal samples were collected (131 treated, 139 control). The average number of faecal samples collected per female was 14.9 (range: 7-21).

Statistical Analysis

First, we evaluated the efficacy of the anthelmintic treatment. To do this, we compared strongyle nematode and lungworm counts between treated and control females both prior and after applying the treatment. We log-transformed the parasite data to meet the assumptions of normality. For the pre-treatment analysis, which used data from faecal samples collected at capture, we tested for differences in parasite counts between the two treatment groups using analysis of variance (ANOVA). For the treatment analysis, we accounted for repeated observations by using linear mixed models (LMM) with Animal ID included as a random effect. As fixed effects, we included treatment, treatment period, and the interaction between these two terms. We divided the study period into two distinct time periods based on the expected duration of the drug treatment (Papadopoulos et al. 2009): a 120 day 'treatment' period and a 'post-

treatment' period during which the effects of the drug wore off (>120 days after treatment). We used the treatment x treatment period interaction in all models to capture changes in anthelmintic drug efficacy through time. We also included a series of covariates in the LMMs including: age, group size and season (wet vs. dry). Each observation month was classified as either wet or dry using monthly rainfall records from the study site. Wet months (June-November 2011, April 2012) averaged 113.2 mm of rainfall, while dry months (December 2011-March 2012) averaged 12.4 mm.

Next, we examined the effects of treatment on female time budgets by comparing the time spent by treated and control females in each behaviour category (foraging, resting, moving, vigilance and other behaviours. We used JWatcher (Blumstein & Daniel 2007) to convert the behavioural voice recordings into time budgets summarising the proportion of time each female devoted to each behaviour. We used Wilcoxon signed-rank tests to evaluate differences in behaviour between treatment groups separately for the treatment and post-treatment periods.

Finally, to determine whether parasite removal had individual-level effects on behavioural time allocation we used multivariate models to test for effects of treatment on the ratio of time females spent foraging vs. engaging in other activities. We based our behaviour ratio analyses around foraging because time allocation to this behavior has been linked to nematode parasitism in livestock (Kyriazakis et al. 1998; Coop & Kyriazakis 1999; Forbes et al. 2007), and because we observed an effect of anthelmintic treatment on foraging in our own time budget analysis. We calculated four behaviour ratios as indicators of potential changes in time allocation to competing activities with foraging: (1) foraging: vigilance, (2) foraging: resting, (3) foraging: moving, and (4) foraging: other. Ratios were calculated only for observations where individuals performed both behaviours of interest. For each observation, the proportion of time

allocated to all behaviours sums to one, therefore our ratios reflect the relative proportion of time, per observation, invested in foraging over other activities. As such, the ratios provide a description of time allocation decisions made by individuals in each specific observation bout. We used LMMs with Animal ID included as a random effect to account for repeated measures. Fixed effects included treatment, treatment period, and the interaction between the two terms. We also included several key covariates in the LMMs, including time of day, age, group size, and season. Initial models included the interactions between treatment and each covariate, but because none of these variables emerged as significant predictors of behavioural ratios we removed them from the final models. All behaviour ratios were log transformed and model residuals were tested for normality. Statistical analyses were performed in JMP 4.0.2 (SAS Institute 2000). Results were considered significant at $P \leq 0.05$.

RESULTS

Treatment Effect on Parasite Load

Prior to anthelmintic treatment, there was no difference between treated and control females in either strongyle nematode or lungworm counts (ANOVA, strongyle: $F_{1,17} = 0.36$, $P = 0.56$; lungworm: $F_{1,17} = 0.0005$, $P = 0.98$; Fig 2.1). After treatment, we found that treated females shed significantly fewer strongyle eggs and lungworm larvae than did control females as evidenced by a main effect of treatment (Table 2.1). However, the difference between treatment groups disappeared after the 120 day treatment period and we found significant treatment by treatment period interaction for both parasite taxa. Treated females shed fewer strongyle egg and lungworm larvae than control females but only during the treatment period (≤120 days post treatment) and not the post-treatment period (>120 days post treatment, Table 2.1, Fig 2.1).

Neither age, group size, nor season had significant effects on strongyle and lungworm counts (Table 2.1).

Gazelle Daily Activity and Effects of Treatment on Time Budgets

On average, untreated, naturally infected (control) females devoted 28% of their time to foraging, 31% to resting, 21% to moving, 16% to vigilance, and 3% to other activities. Treated females spent significantly more time foraging than did controls during the treatment period (Wilcoxon signed-rank test, $z = -2.21$, $N = 18$, $P = 0.03$, Fig. 2.2a), but this effect disappeared during the post-treatment period ($z = -1.01$, $N = 17$, $P = 0.31$, Fig. 2.2b). There was also a significant effect of treatment on vigilance during the treatment, but not the post-treatment period (treatment period: $z = 2.3$, $P = 0.02$, Fig. 2.2a; post-treatment period: $z = 1.3$, $P = 0.2$; Fig. 2.2b). Treatment had no significant effect on any other behaviour during either period (treatment period, resting: $z = 1.15$, $P = 0.25$, moving: $z = -0.88$, $P = 0.38$, other: $z = 0.44$, $P = 0.66$, Fig. 2.2a; post-treatment period, resting: $z = 0.14$, $P = 0.89$, moving: $z = -0.05$, $P = 0.96$, other: $z = -1.01$, $P = 0.31$; Fig. 2.2b).

Treatment Effects on Behavioural Time Allocation

When examining the relative proportion of time females invested in foraging compared to competing activities we found that treated females devoted more time to foraging over vigilance than did control females. While this effect was present during the treatment period, it disappeared during the post-treatment period as illustrated by a significant treatment by treatment period interaction (Table 2.2). There was no effect of treatment on the ratio of foraging to any other behaviour (Table 2.2).

In addition to parasite treatment, group size and season were also significant predictors of behavioural time allocation in female gazelles. Group size was a significant predictor of the

foraging to vigilance and foraging to resting ratios. Females allocated more time to foraging over both vigilance and resting when they were in larger groups (Table 2.2). Season was a predictor of the foraging to moving ratio; females allocated more time to moving over foraging during the wet season (Table 2.2).

DISCUSSION

Several factors can affect how much time animals allocate to competing activities. Here, we show that parasite removal influenced time allocation to core activities in female Grant's gazelles. Over ninety percent of female gazelle daily activity was devoted to four key behaviours: foraging (28%), resting (31%), moving (21%), and vigilance (16%). Other behaviours such as reproductive, agonistic, and maintenance behaviours (e.g. grooming) comprised less than 4% of daily activity. Females relieved of gastrointestinal and pulmonary nematodes adjusted their daily time budgets. At the group level, parasite removal was associated with an increase in foraging and a decrease in vigilance. This effect was also apparent at the individual level. During individual focal observations, treated females allocated more time to foraging at the expense of vigilance suggesting that parasites directly affect host time allocation decisions.

The pattern of daily activity observed for female Grant's gazelles is similar to what has been reported for other wild bovids (Neuhaus & Ruckstuhl 2002; Hamel & Cote 2008; Smith & Cain 2009). Importantly, we found that anthelmintic treatment disrupted these activity patterns. In particular, treatment affected the relative amount of time females allocated to foraging. During the treatment period, treated females spent a significantly larger proportion of their time foraging compared to controls. However, during the post-treatment period the difference disappeared such that treated and control females spent similar amounts of time foraging. The difference in

foraging behaviour between treated and control females during the treatment period, and the disappearance of this effect during the post-treatment period strongly suggest that parasites are directly responsible for the differences in female foraging behaviour. Furthermore, the foraging effect observed in gazelles is consistent with previous studies on domestic ruminants showing that nematode infection can induce reductions in forage intake (Fox 1997; Kyriazakis et al. 1998; Forbes et al. 2000; Gunn & Irvine 2003). Nematode-induced suppression of feeding in ruminants is thought to be associated with immune and endocrine responses that can lead to the release of appetite inhibitors or the suppression of appetite enhancers (Fox et al. 1989; Greer et al. 2005; Forbes 2008; Zaralis et al. 2008).

Treated and control females also differed in the relative proportion of time devoted to vigilance. Treated females allocated less time to vigilance than control females during the treatment period but not during the post-treatment period. The fact that treated females increased time invested in foraging and simultaneously decreased time invested in vigilance suggests that individuals without nematode infections may have substituted vigilance for foraging. This idea is supported by our behaviour ratio models which show that at the level of individual focal observations, treated females increased foraging time at the expense of vigilance. The mean foraging to vigilance ratio for treated females was 10.7 compared to 3.4 for control females, indicating that on average treated females allocated over 3 fold more time foraging over vigilance during a typical 20 minute focal observation. This suggests that parasite removal induced a major re-allocation of time in gazelles. The fact that the difference between treated and control females disappeared during the post-treatment period further implicates parasite removal as a causal factor driving the time re-allocation. Once the treatment wore off, the foraging to vigilance ratios for treated vs. control females were 8.3 compared to 5.6.

It is noteworthy that time re-allocation associated with parasite treatment and foraging behaviour was observed for vigilance and no other behaviour. Investing time in anti-predator behaviour is crucial for survival, but time allocated to vigilance incurs a cost since it interrupts active foraging. For this reason, inherently vulnerable individuals are predicted to invest more time in anti-predator behaviours compared to individuals that are less vulnerable to predation (Elgar 1989). Predator vulnerability is often linked to individual traits such as age, sex, reproductive status and body condition, and a number of studies suggest that these traits can determine how animals allocate their time between foraging and vigilance behaviours (Elgar 1989; Rieucau & Martin 2008; Edwards et al. 2013). In European rabbits (*Oryctolagus cuniculus*), for example, females increase vigilance time at the expense of foraging during late pregnancy possibly because of their reduced capacity to evade predators (Monclus Rodel 2009). Similarly, yellow-bellied marmots (*Marmota flaviventris*) in poor body condition at the end of the summer invest more time in foraging activities at the expense of vigilance to enhance overwinter survival (Lea & Blumstein 2011). Both examples suggest that aspects of an animal's physical condition can influence the relative time allocation to foraging versus vigilance. The fact that parasite removal significantly altered foraging to vigilance ratios in Grant's gazelles suggests that parasites can also act as important drivers of behavioural time allocation decisions. Since treated females re-allocated time spent vigilant to foraging, it seems likely that parasite removal reduced female vulnerability to predators. One way the change in vulnerability could have occurred is if treated females were in better condition and thus better able to escape predators. These 'good condition' females may therefore have the flexibility to invest less time in anti-predator activities with no negative consequences. We did not have data on female body condition in this study, however one interesting direction for future studies could be to examine

how parasites induce changes in body condition and affect time allocation to foraging and anti-predator behaviours.

In addition to treatment, group size influenced time allocation to foraging and vigilance in gazelles. Specifically, females in larger groups allocated more time to foraging at the expense of vigilance. A re-allocation of time towards foraging and away from vigilance with increasing group size has been observed in many other taxa ranging from birds to mammals (Blumstein et al. 2003; Childress & Lung 2003; Hopewell et al. 2005; Watson et al. 2007; Lashley et al. 2014). Such effects of group size on vigilance are thought to be a consequence of the presence of 'more eyes' (Pulliam 1973), where animals in larger groups benefit from increased group vigilance. Another possibility is that individual time investment in vigilance declines in larger groups because of the decline in individual predation risk via a dilution effect (Hamilton 1971). More generally, group size and season emerged as significant predictors of gazelle time allocation to foraging relative to other activities such as resting and moving. These results highlight the role of social and environmental factors in shaping individual time allocation.

There is considerable interest in understanding how social, ecological and environmental factors influence variation in an animal's behavioural repertoire and the fitness consequences of these behavioural differences. For example, predation studies have shown that exposure to predators can result in a major re-allocation of prey behaviours (Creel et al. 2005), highlighting the importance of natural enemies in shaping individual behaviour. Our work demonstrates that another type of natural enemy, parasites, can also affect host time allocation decisions. Specifically, we found that female gazelles relieved of gastrointestinal and pulmonary nematodes increased time invested in foraging at the expense of vigilance. In our study, group size also predicted how much time individuals invested in foraging versus vigilance, and interestingly the

magnitude of the parasite treatment effect was of similar size to the effect observed for group size (F = 7.0 *vs.* 5.1). This suggests that parasites can induce changes in host behaviour that are equivalent in magnitude to some of the most commonly studied social drivers of behavioural variation, reinforcing the importance of parasitism as a key process shaping animal behaviour in the wild.

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REFERENCES

Alberts, S. C., Hollister-Smith, J. A., Mututua, R. S., Sayialel, S. N., Muruthi, P. M., Warutere, J. K., & Altmann, J. (2005). Seasonality and long-term change in a savanna environment. *Seasonality in Primates: Studies of Living and Extinct Human and Non-Human Primates, 44*, 157-195. doi: 10.1017/cbo9780511542343.007

Altmann, J. (1974). Observational Study of Behavior: Sampling Methods. *Behaviour, 49*, 227-267.

Arneberg, P., Folstad, I., & Karter, A. J. (1996). Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology, 112*, 213-219.

Bachman, G. C. (1993). The Effect of Body Condition on the Trade-Off Between Vigilance and Foraging in Belding Ground-Squirrels. *Animal Behaviour, 46*(2), 233-244. doi: 10.1006/anbe.1993.1185

Blumstein, D. T., Daniel, J. C., & Sims, R. A. (2003). Group size but not distance to cover influences agile wallaby (Macropus agilis) time allocation. *Journal of Mammalogy, 84*(1), 197-204. doi: 10.1644/1545-1542(2003)084<0197:gsbndt>2.0.co;2

Blumstein, D. T. & Daniel, J. C. (2007). *Quantifying Behavior the JWatcher Way*. Sunderland, Massachusetts: Sinauer.

Brivio, F., Grignolio, S., Brambilla, A., & Apollonio, M. (2014). Intra-sexual variability in feeding behaviour of a mountain ungulate: size matters. *Behavioral Ecology and Sociobiology, 68*(10), 1649-1660.

Childress, M. J., & Lung, M. A. (2003). Predation risk, gender and the group size effect: does elk vigilance depend upon the behaviour of conspecifics? *Animal Behaviour, 66*, 389-398. doi: 10.1006/anbe.2003.2217

Coop, R. L., & Holmes, P. H. (1996). Nutrition and parasite interaction.*International journal for parasitology*, *26*(8), 951-962.

Coop, R. L., & Kyriazakis, I. (1999). Nutrition-parasite interaction. *Veterinary Parasitology, 84*(3-4), 187-204. doi: 10.1016/s0304-4017(99)00070-9

Creel, S., Winnie, J., Maxwell, B., Hamlin, K., & Creel, M. (2005). Elk alter habitat selection as an antipredator response to wolves. *Ecology, 86*(12), 3387-3397. doi: 10.1890/05-0032.

Creel, S., Schuette, P., & Christianson, D. (2014). Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community.*Behavioral Ecology*, aru050

Cresswell, W. (1994). Flocking is an effective anti-predation strategy in redshanks, Tringa totanus. *Animal behaviour, 47*(2), 433-442.

Dunbar, R. I. M., Korstjens, A. H., & Lehmann, J. (2009). Time as an ecological constraint. *Biological Reviews, 84*(3), 413-429. doi: 10.1111/j.1469-185X.2009.00080.x

Edwards, A. M., Best, E. C., Blomberg, S. P., & Goldizen, A. W. (2013). Individual traits influence vigilance in wild female eastern grey kangaroos. *Australian Journal of Zoology, 61*(4), 332. doi: 10.1071/zo13025

Elgar, M. A. (1989). Predator Vigilance and Group Size in Mammals and Birds - A Critical Review of the Emperical Evidence. *Biological Reviews of the Cambridge Philosophical Society, 64*(1), 13-33. doi: 10.1111/j.1469-185X.1989.tb00636.x

Ezenwa, V. O. (2003). Habitat overlap and gastrointestinal parasitism in sympatric African bovids. *Parasitology, 126*(4), 379-388. doi: 10.1017/s0031182002002913

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology*, *15*(3), 446-454. doi: 10.1093/beheco/arh028

Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology, 26*(1), 123-133. doi: 10.1111/j.1365-2435.2011.01919.x

Fenner, A. L., & Bull, C. M. (2008). The impact of nematode parasites on the behaviour of an Australian lizard, the gidgee skink Egernia stokesii. *Ecological Research, 23*(5), 897-903. doi: 10.1007/s11284-007-0453-1

Forbes, A. B., Huckle, C. A., Gibb, M. J., Rook, A. J., & Nuthall, R. (2000). Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young

grazing cattle. *Veterinary Parasitology, 90*(1-2), 111-118. doi: 10.1016/s0304-4017(00)00218-1

Forbes, A. B., Huckle, C. A., & Gibb, M. J. (2004). Impact of eprinomectin on grazing behaviour and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. *Vet Parasitol, 125*(3-4), 353-364. doi: 10.1016/j.vetpar.2004.07.025

Forbes, A. B., Huckle, C. A., & Gibb, M. J. (2007). Evaluation of the effect of eprinomectin in young dairy heifers sub-clinically infected with gastrointestinal nematodes on grazing behaviour and diet selection. *Vet Parasitol, 150*(4), 321-332. doi: 10.1016/j.vetpar.2007.09.031

Forbes, A. (2008). *Grazing Behaviour, Inappetence and Production Losses in Cattle with Sub-clinical Parasitic Gastroenteritis* (Doctoral dissertation, Ghent University).

Forrester, S. G., & Lankester, M. W. (1997). Extracting protostrongylid nematode larvae from ungulate feces. *Journal of Wildlife Diseases, 33*(3), 511-516.

Fox, M. T. (1997). Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. *Veterinary Parasitology, 72,* 285-308.

Fox, M. T., Gerrelli, D., Pitt, S. R., Jacobs, D. E., Gill, M., & Gale, D. L. (1989). Ostertagia Ostertagi Infection in the Calf - Effects of a Trickle Challenge on Appetite, Digestibility, Rate of Passage of Digesta and Liveweight Gain. *Research in Veterinary Science, 47*(3), 294-298.

Frid, A. (1997). Vigilance by female Dall's sheep: interactions between predation risk factors. *Animal Behaviour, 53*(4), 799-808.

Fuller, R. A., Bearhop, S., Metcalfe, N. B., & Piersma, T. (2013). The effect of group size on vigilance in Ruddy Turnstones Arenaria interpres varies with foraging habitat. *Ibis, 155*(2), 246-257.

Geist, V. (1971). *Mountain sheep*. University of Chicago Press, Chicago, Illinois, USA

Gélin, U., Wilson, M. E., Coulson, G. M., & Festa-Bianchet, M. (2013). Offspring sex, current and previous reproduction affect feeding behaviour in wild eastern grey kangaroos. *Animal Behaviour, 86*(5), 885-891. doi: 10.1016/j.anbehav.2013.08.016

Greer, A. W., Stankiewicz, M., Jay, N. P., McAnulty, R. W., & Sykes, A. R. (2005). The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite Trichostrongylus colubriformis on food intake and utilization in both immunologically naive and competent sheep. *Animal Science, 80*, 89-99.

Gunn, A. I., R. J. . (2003). Subclinical Parasitism and Ruminant Foraging Strategies: A Review. *Wildlife Society Bulletin, 31*, 117-126.

Halupka, K., & Osińska-Dzienniak, A. (2013). Group foraging by wintering rooks: combined effects of safety and energy requirements. *Behaviour, 150*(3-4), 381-395.

Hamel, S., & Côté, S. D. (2008). Trade-offs in activity budget in an alpine ungulate: contrasting lactating and nonlactating females. *Animal Behaviour, 75*(1), 217-227. doi: 10.1016/j.anbehav.2007.04.028

Hamilton, W. D. (1971). Geometry for the selfish herd. *Journal of theoretical Biology, 31*(2), 295-311.

Hopewell, L., Rossiter, R., Blower, E., Leaver, L., & Goto, K. (2005). Grazing and vigilance by Soay sheep on Lundy island: Influence of group size, terrain and the distribution of vegetation. *Behavioural Processes, 70*(2), 186-193. doi: 10.1016/j.beproc.2005.04.009

Huebinger RM, De Maar TW, Woodruff LH, Pomp D, Louis EE (2006) Characterization of eight microsatellite loci in Grant's gazelle(*Gazellagranti*). Molecular Ecology Notes 6: 1150-1151.

Illius, A. W., Duncan, P., Richard, C., & Mesochina, P. (2002). Mechanisms of functional response and resource exploitation in browsing roe deer. *Journal of Animal Ecology, 71*(5), 723-734. doi: 10.1046/j.1365-2656.2002.00643.x

Kotler, B. P., Gross, J. E., & Mitchell, W. A. (1994). Applying patch use to assess aspects of foraging behavior in Nubian ibex. *The Journal of wildlife management*, 299-307.

Key, C., & Ross, C. (1999). Sex differences in energy expenditure in non-human primates. *Proceedings of the Royal Society B-Biological Sciences, 266*(1437), 2479-2485.

Kingdon J, Hoffmann M (2013) Mammals of Africa: Pigs, Hippopotamuses, Chevrotain, Giraffes, Deer and Bovids (Vol. 6). A&C Black.

Kyriazakis, I., Tolkamp, B. J., & Hutchings, M. R. (1998). Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour, 56*, 265-274. doi: 10.1006/anbe.1998.0761

Lashley, M. A., Chitwood, M. C., Biggerstaff, M. T., Morina, D. L., Moorman, C. E., & DePerno, C. S. (2014). White-tailed deer vigilance: the influence of social and environmental factors. *PLoS One, 9*(3), e90652. doi: 10.1371/journal.pone.0090652

Lea, A. J., & Blumstein, D. T. (2011). Age and sex influence marmot antipredator behavior during periods of heightened risk. *Behav Ecol Sociobiol, 65*(8), 1525-1533. doi: 10.1007/s00265-011-1162-x

Main, M. B. (2008). Reconciling competing ecological explanations for sexual segregation in ungulates. *Ecology, 89*(3), 693-704. doi: 10.1890/07-0645.1

Michelena, P., & Deneubourg, J. L. (2011). How group size affects vigilance dynamics and time allocation patterns: the key role of imitation and tempo.*Plos One*, *6*(4), e18631.

Monclús, R., & Rödel, H. G. (2009). Influence of Different Individual Traits on Vigilance Behaviour in European Rabbits. *Ethology*, *115*(8), 758-766. doi: 10.1111/j.1439-0310.2009.01661.x

Neuhaus, P., & Ruckstuhl, K. E. (2002). Foraging behaviour in Alpine ibex (Capra ibex): consequences of reproductive status, body size, age and sex. *Ethology Ecology & Evolution, 14*(4), 373-381. doi: 10.1080/08927014.2002.9522738

O'BryhimJ,Somers C, Lance SL, Yau M, Boreham DR, Jones KL, Taylor EB (2013) Development and characterization of twenty-two novel microsatellite markers for the mountain whitefish, *Prosopiumwilliamsoni* and cross-amplification in the round whitefish, *P. cylindraceum*, using paired-end Illumina shotgun sequencing. Conservation Genetics Resources 5:89-91.

Papadopoulos, E., Fragkou, I. A., Mavrogianni, V. S., Gougoulis, D. A., Orfanou, D. C., Gallidis, E., ... & Fthenakis, G. C. (2009). Persistent efficacy of long-acting moxidectin for control of trichostrongylid infections of sheep. *Small Ruminant Research*, *81*(2), 171-173.

Pelletier, F., & Festa-Bianchet, M. (2004). Effects of body mass, age, dominance and parasite load on foraging time of bighorn rams, Ovis canadensis. *Behavioral Ecology and Sociobiology, 56*(6), 546-551. doi: 10.1007/s00265-004-0820-7

Powolny, T., Bretagnolle, V., Aguilar, A., & Eraud, C. (2014). Sex-related differences in the trade-off between foraging and vigilance in a granivorous forager. *PloS one*, *9*(7), e101598.

Prates, H. M.,& Bicca-Marques, J. C. (2008). Age-sex analysis of activity budget, diet, and positional behavior in Alouatta caraya in an orchard forest. *International Journal of Primatology, 29*(3), 703-715. doi: 10.1007/s10764-008-9257-6

Pulliam, H. R. (1973). On the advantages of flocking. *Journal of theoretical Biology, 38*(2), 419-422.

Rauter, C. M., & Moore, A. J. (2004). Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Animal Behaviour, 68*(4), 695-702. doi: 10.1016/j.anbehav.2003.09.018

Rieucau, G., & Martin, J. G. A. (2008). Many eyes or many ewes: vigilance tactics in female bighorn sheep Ovis canadensis vary according to reproductive status. *Oikos, 117*(4), 501-506. doi: 10.1111/j.2007.0030-1299.16274.x

Ruckstuhl, K. E., Festa-Bianchet, M., & Jorgenson, J. T. (2003). Bite rates in Rocky Mountain bighorn sheep (Ovis canadensis): effects of season, age, sex and reproductive status. *Behavioral Ecology and Sociobiology, 54*(2), 167-173. doi: 10.1007/s00265-003-0615-2

SAS Institute (2000). J*MP Statistics and Graphics Guide*. Version 4.0.2.

Sibbald, A. M., & Hooper, R. J. (2004). Sociability and the willingness of individual sheep to move away from their companions in order to graze. *Applied Animal Behaviour Science, 86*(1-2), 51-62. doi: 10.1016/j.applanim.2003.11.010

Smith, S. M., & Cain, J. W., III. (2009). Foraging efficiency and vigilance behaviour of impala: the influence of herd size and neighbour density. *African Journal of Ecology, 47*(1), 109-118. doi: 10.1111/j.1365-2028.2008.01014.x

Spinage, C. A. (1976). Age Determination of the Female Grant's Gazelle. *East African Wildlife Journal, 14*, 121-134.

Stearns, S. C. (1992). *The evolution of life histories*. – Oxford University Press, New York

Tadesse, S. A., & Kotler, B. P. (2014). Effects of habitat, group-size, sex-age class and seasonal variation on the behavioural responses of the mountain nyala (Tragelaphus buxtoni) in Munessa, Ethiopia. *Journal of Tropical Ecology*,*30*(01), 33-43.

Tettamanti, F., & Viblanc, V. A. (2014). Influences of Mating Group Composition on the Behavioral Time-Budget of Male and Female Alpine Ibex (Capra ibex) during the Rut. *PloS one*, *9*(1), e86004.

Walther, F. R. (1969). Flight behaviour and avoidance of predators in Thomson's gazelle (Gazella thomsoni Guenther 1884). *Behaviour*, *34*(3), 184-220.

Walther, F. R. (1973). Round-the-clock Activity of Thomson's Gazelle (Gazella thomsoni Gunther 1884) in the Serengeti National Park. *Zeitschrift für Tierpsychologie*, *32*(1), 75-105.

Walther FR, Mungall EC, Grau GA (1983) Gazelles and their relatives. A study in territorial behavior. Park Ridge, New Jersey: Noyes.

Watson, M., Aebischer, N. J., & Cresswell, W. (2007). Vigilance and fitness in grey partridges Perdix perdix: the effects of group size and foraging-vigilance trade-offs on predation mortality. *J Anim Ecol*, *76*(2), 211-221. doi: 10.1111/j.1365-2656.2006.01194.x

Witter, L. A., Johnson, C. J., Croft, B., Gunn, A., & Gillingham, M. P. (2012). Behavioural trade-offs in response to external stimuli: time allocation of an Arctic ungulate during varying intensities of harassment by parasitic flies. *J Anim Ecol*, *81*(1), 284-295. doi: 10.1111/j.1365-2656.2011.01905.x

Zaralis, K., Tolkamp, B. J., Houdijk, J. G. M., Wylie, A. R. G., & Kyriazakis, I. (2008). Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. *Journal of Animal Science*, *86*(8), 1891-1903. doi: 10.2527/jas.2007-0698

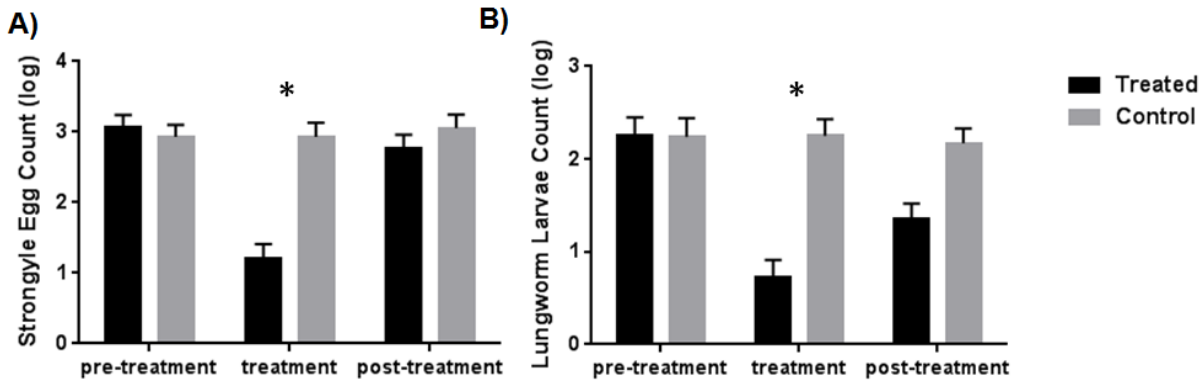

Figure 2.1: A) Mean (± SE) strongyle egg count (log) and **B)** lungworm larvae count (log) for treated (black boxes) and control females (gray boxes) during each treatment period. Statistically significant differences ($P \leq 0.05$) are depicted with an asterisk.

Table 2.1
Predictors of strongyle nematode and lungworm counts in female Grant's gazelle. Significant effects are in bold. Reference levels are indicated in brackets.

Variables		Strongyle eggs			Lungworm larvae		
		N = 251			*N* = 251		
	DF	*Estimate*	*F*	*P*	*Estimate*	*F*	*P*
Treatment [Treated]	1	**-0.5**	**15.11**	**0.002**	**-0.59**	**27.47**	**<0.0001**
Treatment period [Post]	1	**0.42**	**53.29**	**<0.0001**	**0.13**	**6.93**	**0.009**
Treatment [T] x T. period [Post]	1	**0.35**	**86.21**	**<0.0001**	**0.18**	**27.13**	**<0.0001**
Age	1	0.02	2.71	0.11	0.01	1.04	0.31
Group size	1	-0.003	0.06	0.8	-0.008	0.47	0.49
Season [dry]	1	0.02	0.11	0.74	-0.06	1.56	0.21

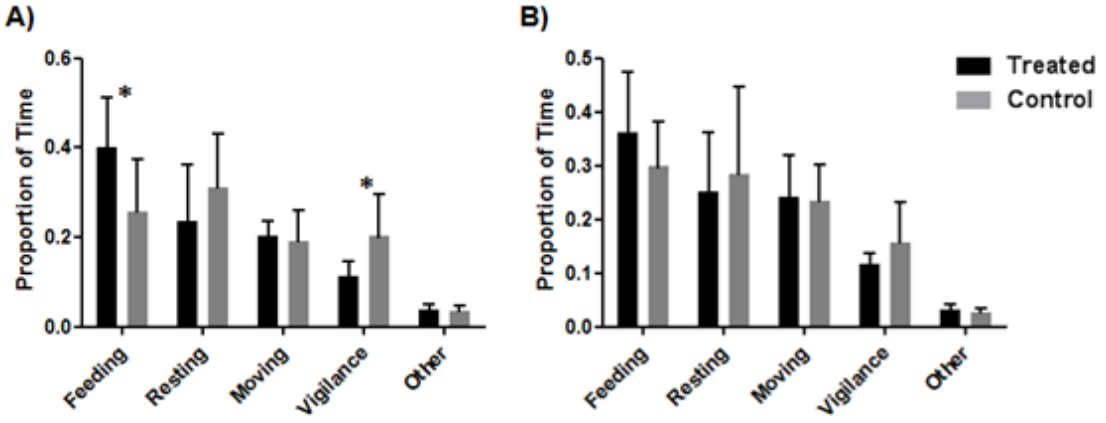

Figure 2.2: Female Grant's gazelle activity (± SD) by treatment group during the **A)** treatment period (≤120 days after applying the treatment) and **B)** post-treatment period (>120 days after applying the treatment). Statistically significant differences (P≤ 0.05) and depicted with an asterisk.

Table 2.2
Predictors of relative time allocation to foraging vs. other activities in female gazelles. Significant effects are in bold. Reference levels are indicated in brackets.

Variables		Foraging: Vigilance *N* = 424			Foraging: Resting *N* = 396			Foraging: Moving *N* = 427			Foraging: Other *N* = 382		
	DF	*Estimate*	*F*	*P*	*Estimate*	*F*	*P*	*Estimate*	*F*	*P*	*Estimate*	*F*	*P*
Treatment [Treated]	1	**0.15**	**7.0**	**0.02**	0.06	0.34	0.57	0.06	1.45	0.25	0.04	0.61	0.44
Treatment period [Post]	1	0.008	0.03	0.86	-0.01	0.04	0.84	0.002	0.002	0.97	0.03	0.34	0.56
Treatment [T] x T. period [Post]	1	**-0.06**	**3.93**	**0.05**	0.003	0.004	0.95	-0.004	0.02	0.9	-0.05	1.67	0.2
Age	1	0.006	0.62	0.44	0.001	0.006	0.94	-0.003	0.2	0.66	-0.005	0.41	0.53
Group Size	1	**0.02**	**5.15**	**0.02**	**0.04**	**5.65**	**0.02**	0.001	0.02	0.89	0.008	0.59	0.44
Season [dry]	1	-0.07	3.24	0.07	-0.06	0.83	0.36	**-0.07**	**4.03**	**0.04**	0.05	1.14	0.29
Time of Day	3	-	1.52	0.21	-	1.24	0.3	-	1.16	0.32	-	2.37	0.07

CHAPTER 3

HETEROZYGOSITY-FITNESS CORRELATIONS ARE CONTEXT DEPENDENT IN THE WILD[2]

[2]Katherine E. L. Worsley-Tonks, Stacey Lance, Vanessa O. Ezenwa. To be submitted to *Journal of Evolutionary Biology*.

ABSTRACT

Heterozygosity-fitness correlations (HFCs) are widely used to explore the effects of inbreeding in wild populations. However, the biological significance of HFCs has been the subject of intense debate, and it has recently been suggested that the magnitude and direction of these correlations may be context-dependent (e.g. vary with different host characteristics or environmental conditions). We tested this hypothesis in a free-ranging population of Grant's gazelles (*Nanger granti*) in Kenya. Specifically, we tested for associations between standard multilocus heterozygosity and parasitic worm infections, and examined how these relationships varied with animal age, sex and environmental context (e.g. seasonality). We did this using two approaches: a cross-sectional approach focusing on 103 individuals sampled at a single time point, and a longitudinal approach focusing on a subset of 25 individuals sampled over 12 months. For both types of analyses we found that age and environment determined the magnitude of heterozygosity-fitness correlations, but the relevant context differed for different parasite taxa. Our work demonstrates that individuals that are less genetically diverse may be more likely to suffer the consequences of infection, but only under certain conditions and at certain times.

INTRODUCTION

Expanding human activities and heterogeneously distributed resources are causing animals to live in increasingly small and fragmented populations. One concern is that these populations may be more susceptible to demographic and environmental stochasticity (Lande 1988). One of these demographic effects is an increase in mating between relatives, or

inbreeding, which can have a range of negative consequences for individuals and populations (Crow 1948; Charlesworth & Charlesworth 1987; Crnokrak & Roff 1998; Frankham 2003). At the individual-level, inbreeding depression can manifest itself in multiple ways, such as through low recruitment, birth rate, and increased susceptibility to infectious diseases (Coulson et al. 1998; Coltman et al. 1998; Coltman et al. 1999; Keller & Waller 2002; Kruuk et al. 2002; Acevedo-Whitehouse et al. 2003), all of which can contribute to reductions in survival and reproductive success. These inbreeding-associated fitness consequences have also been linked to population declines (Westemeier et al. 1998; Nieminen et al. 2001; Acevedo-Whitehouse et al. 2005), and in some cases, even local extinction (Frankham 1995; Lynch et al. 1995; Saccheri et al. 1998).

One widespread approach of studying the effects of inbreeding in natural populations is by testing for heterozygosity-fitness correlations (HFCs, Balloux et al. 2004). Studies exploring HFCs often examine relationships between individual heterozygosity estimated from molecular markers and traits related to performance or fitness such as reproductive success, birth rate, body size, rate of development, survival, and parasite susceptibility (Coltman et al. 1999; Slate et al. 2000; Slate & Pemberton 2002; Acevedo-Whitehouse et al. 2005; Charpentier et al. 2008; Ryder et al. 2009; Haanes et al. 2013). Although HFCs have frequently been observed in wild populations (Chapman et al. 2009), these associations can arise for several reasons, and may only reflect effects of inbreeding when the measure of genetic variability represents genome-wide heterozygosity (general effect hypothesis, Hansson & Westerberg 2002; Szulkin et al. 2010). Alternatively, HFCs may occur if the chosen markers are in genes under selection with direct fitness advantages (direct effect hypothesis, David 1998), or if they are linked with genes under

selection (local effect hypothesis, Lynch & Walsh 1998; David 1998; Hansson & Westerberg 2002).

Although our understanding of the underlying mechanisms causing HFCs has improved in recent years (Szulkin et al. 2010; Kardos et al. 2014; Miller et al. 2014), the biological significance of HFCs is still under intense debate, in part, because the detection of HFCs in nature is notoriously inconsistent. For example, in a recent meta-analysis, Chapman et al. (2009) found that although HFCs arise frequently in wild populations, associations are relatively weak and can be inconsistent from one population or taxon to the next. Indeed, several recent studies show that even in the same system, the direction and magnitude of HFCs can vary with context. For example, correlations might only be detected under certain environmental conditions, either when resources are limited (Lens et al. 2000; Lesbarreres et al. 2005) or conversely, when conditions are favorable (Harrison et al. 2011; Annavi et al. 2014). The magnitude and direction of HFCs may also vary between different demographic groups. For example, in the sexually dimorphic lizard, *Takydromus viridipunctatus*, infestation with trombiculid mites declined with heterozygosity in males but not females (Shaner et al. 2013), indicating sex-specific HFCs. This emphasizes the fact that non-genetic factors influence HFCs, and that context may help explain the variability with which these patterns are detected.

In this study, we tested for context-dependent HFCs in a population of Grant's gazelles (*Nanger granti*) in the Laikipia District of Central Kenya. Grant's gazelles are estimated to number approximately 350,000 individuals (East 1999), and are distributed across East Africa from Ethiopia and south Sudan, across Kenya, and into Uganda and Tanzania. In Kenya, three highly divergent Grant's gazelle lineages have been described with no evidence of gene flow between them, suggesting that despite close geographic proximity between populations extensive

reproductive isolation can occur (Lorenzen et al. 2008). In addition, pressures such as the expansion of agriculture, habitat degradation, and food competition with livestock have caused Grant's gazelle populations to decline over large parts of their range (IUCN 2008). As a result, local populations of this species are shrinking (IUCN 2008) and may be becoming increasingly isolated.

Given the demographic structure of Grant's gazelle populations (Lorenzen et al. 2008; IUCN 2008), we used this species as a model to investigate potential inbreeding effects on fitness via HFCs. To do this, first, we evaluated whether a panel of microsatellite markers reflected genome-wide effects on HFCs by estimating the amount of identity disequilibrium (ID) among markers. ID measures the covariance in heterozygosity across markers (David 1998; David 2007), and the variance in inbreeding within the focal population (Szulkin et al. 2010; Kardos et al. 2014). When ID is present the markers under consideration are more likely to reflect genome-wide effects (Miller & Coltman 2014). Next, we explored associations between individual heterozygosity, calculated from our set of microsatellites, and parasite infection, and tested whether these relationships varied with individual age, sex and environmental conditions (e.g. seasonality). Parasites can negatively impact the fitness of their hosts by reducing growth and development rates, body mass, condition, survival and reproduction (Hurtrez-Bousses et al. 1997; Ujvari & Madsen 2006; Sperry et al. 2009; Hillegrass et al. 2010), and as such, have been the target of several HFC studies (e.g. Coltman et al. 1999; Acevedo-Whitehouse et al. 2003; Hawley et al. 2005; Rijks et al. 2008; Voegeli et al. 2012). Here, we used cross-sectional and longitudinal sampling approaches to explore the context-dependency of heterozygosity-parasite correlations. For both types of analyses, we predicted that individual heterozygosity would negatively correlate with parasite infection, but we expected these associations to vary with

context. Specifically, we hypothesized that HFCs would be stronger in younger individuals, in males, and under harsher environmental conditions (Coltman et al. 1999; LesBarreres et al. 2005; Rijks et al. 2008; Chapman et al. 2009; Shaner et al. 2013).

METHODS

Study Population

Grant's gazelles were captured and ear-tagged at the Mpala Research Centre (MRC), Laikipia, Kenya (0°17'N, 37°52' E), as part of a long-term study (Ezenwa et al. 2012). MRC is located on 200 km² of unfenced private land surrounded by livestock ranches and wildlife conservancies, and wildlife move freely between MRC and neighboring properties. The current estimated population of Grant's gazelles in Laikipia is 4,700 (J. Ogutu, unpublished data), approximately 200 gazelles reside at MRC. Animals were caught in either August 2009 or June 2011. On both occasions, gazelles were located by helicopter and caught using a hand held net gun fired from an aircraft (Ezenwa et al. 2012). Fecal, blood, and tissue samples were collected for parasitological and genetic analyses. All individuals were also aged at capture. For males, we used a tooth wear criteria for Grant's gazelle described in Stelfox et al. (1985) and took an impression of the upper molar using dental putty (Provil Novo, HerausKulzer). For females, we used an equation that relates horn length to tooth-wear which was developed for a subset of animals from the same population (Ezenwa unpublished data, Ezenwa et al. 2012).

DNA extraction and Genotyping

We genotyped individual gazelles at 12 microsatellite loci developed from individuals from the same population (Worsley-Tonks et al. 2015, Appendix A). Ear tissue samples collected at capture were stored in 95% ethanol until DNA extraction. Whole genomic DNA was extracted from tissue using DNeasy Tissue Kits (QIAGEN Inc, Valencia, CA, USA) according to the

manufacturer's instructions. Microsatellites were amplified using standard polymerase chain reactions (PCR) using a three-primer nested reaction including a universal fluorescently labeled primer to label all reactions (modified from Schuelke 2000). PCRs were performed in a volume of 12.5µl containing 5-10ng of genomic DNA, 2µg bovine serum albumin, 1.5mM $MgCl_2$, 1x PCR gold buffer (Applied Biosystems), 0.36µM universal dye-labeled primer, 0.04 µM tag labeled primer, 0.4µM unlabeled primer, 0.8 mM dNTPs, 4.99µl sterile double-distilled water, and 0.3 units AmpliTaq Gold® Polymerase (Applied Biosystems). All PCR amplifications were performed using an Applied Biosystems GeneAmp 9700, and PCR products were then run on an ABI-3130xl sequencer (Applied Biosystems) and sized with Naurox size standard prepared as described in DeWoody et al. (2004) except that unlabeled primers started with GTTT. Alleles were scored and manually verified using GeneMapper v.3.7 (Applied Biosystems).

Heterozygosity Variation

Observed and expected heterozygosity at each locus were calculated using GeneAlEx v. 6.4 (Peakall & Smouse 2006). We evaluated each locus for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium using GENEPOP v. 4.2, and used Bonferroni corrections to account for multiple tests. We used the program Micro-Checker (Van Oosterhout et al. 2004) to test for null alleles.

We calculated three measures of heterozygosity: standard heterozygosity (SMLH, Coltman et al. 1999), internal relatedness (IR, Amos et al. 2001), and homozygosity weighted by locus (HL, Aparicio et al. 2006) using the program IRmacroN4 (W. Amos, Cambridge University). Since all three metrics were strongly correlated (Pearson's correlation: $P < 0.0001$), we only reported results for SMLH. We estimated identity disequilibrium by calculating g_2 using the program RMES (David et al. 2007). The g_2 statistic depends on the variance of inbreeding of

the population and not locus specific characteristics (David et al. 2007). When there is no variance in inbreeding ($g_2 = 0$), HFCs are not expected to arise (Szulkin et al. 2010).

Parasitological Analyses

For the cross-sectional analyses, fecal samples were collected at capture directly from the rectum of focal individuals. For longitudinal analyses, samples were collected over a 12 month period (July 2011 to June 2012). These samples were collected within 10 minutes of observing a known individual defecate. We screened each fecal sample for five different parasite taxa including: strongyle nematodes (Trichostrongylidae), *Trichuris spp.* (Trichuridae), *Strongyloides spp.* (Strongyloididae), coccidia (Eimeriidae), and lungworms (Trichostrongyloidea). Lungworms were further distinguished into three groups based on tail morphology (Ezenwa et al. 2012). For all taxa except lungworms, we used a modification of the McMaster fecal egg counting technique to quantify parasite egg or oocyst output per gram feces (Ezenwa 2003). For lungworms, we used a modified Baermann method to quantify first stage larvae per 10g of feces (Forrester & Lankester 1997).

We calculated three indices of parasitism: prevalence, intensity, and richness. Prevalence referred to the proportion of hosts infected with at least one individual of a specific parasite taxa. Intensity was defined as the total number of individuals from a single parasite taxon in a host individual (Ezenwa 2003). We measured parasite intensity using fecal egg, oocysts, or larvae counts and reported these measures as egg/oocysts per grams feces (EPG/OPG) and larvae per 10g feces (LPG). Intensity analyses focused on the two dominant parasite groups: strongyles and lungworms. We described parasite richness as the number of different parasite taxa present in a single host. For richness analyses, the three lungworm morph types were counted separately.

Statistical Analysis

Prior to examining heterozygosity-parasite correlations, we assessed whether SMLH varied with gazelle age and sex using general linear models. Next, to investigate associations between individual heterozygosity and parasite infection, and to assess whether these patterns varied with context, we tested the effects of SMLH and its interactions with age, sex, and environmental conditions (capture year for the cross-sectional analyses and season for the longitudinal analyses) on parasite infection. We used three measures of parasite infection as response variables: strongyle intensity, lungworm intensity, and parasite richness.

For the cross-sectional analyses, we log-transformed strongyle intensity and used linear models, and for lungworm intensity we used generalized linear models with a negative binomial distribution and a log-link function. Linear models were also used for parasite richness. In all instances, we included SMLH, age, sex and capture year (2009 or 2011) as main effects, we also included two-way interactions between SMLH and age, sex and capture year. Capture year was included as an environmental measure because 2009 was a drier year than 2011 with a mean annual rainfall of 27.02 mm (range: 0-86.3 mm) compared to 84.7 mm (range: 11-139 mm). We started with a full model (main effects plus all two-way interactions with SMLH) and then determined the minimum adequate model using a stepwise elimination process as described by Crawley (2014). The least significant term was removed at each step and model selection was based on the Akaike's information criterion (AIC). We removed all non-significant interaction terms first and then removed non-significant main effect terms. All factors with $P < 0.1$ were retained in the final model and the model with the lowest AIC value was considered to be the minimum adequate model.

For the longitudinal analyses, we fitted linear mixed-effect models using the "lmer" function in package lme4 (Bates et al. 2011) in R (i386 2.15.1) (RCoreTeam, 2012). Animal ID was included as a random effect in all models to account for repeated measures of the same individuals. SMLH, age, sex, and season were included as main effects, and we also included the two-way interactions between SMLH and age, sex and season. Seasonality was coded as wet or dry based on monthly rainfall records from the study site (wet: July-November 2011 and April-May 2012; dry: December 2011-March 2012 and June 2012). Similar to the cross-sectional models, we used a minimum adequate model approach.

RESULTS

Descriptive Genetic Analyses

Heterozygosity measures were calculated for 103 gazelles (48 captured in 2009 and 56 captured in 2011). None of the 12 loci screened contained null alleles (Table 3.1). After Bonferroni correction, three of twelve loci deviated from HWE (Table 3.1), and were therefore excluded from analyses. Only one pair of loci showed significant linkage disequilibrium (*Nagr44-Nagr48*), and both loci were retained in the final analysis. Average observed and expected heterozygosity were 0.644 (SD = 0.183) and 0.641 (SD = 0.181), respectively. We found evidence of identity disequilibrium, with g_2 being significantly different from 0 ($g_2 \pm$ SD = 0.0325 ±0.015668). Heterozygosity did not vary by sex or age (general linear model: sex: t = -0.25, P = 0.81; age: t = 0.02, P = 0.99).

Individual Heterozygosity and Parasite Infection

A. Cross-sectional analysis

At capture, all study animals were infected with strongyle nematodes and intensity ranged from 100-6,550 EPG (mean = 1516.7). The prevalence of lungworm infection was 96% and

mean intensity ranged from 0-18,561 LPG (mean = 1201.2). Parasite richness ranged from 1 to 7 taxa, with an average of 4.4. Strongyle intensity increased with age and varied by capture year (2011 > 2009), but there was no main effect of SMLH on strongyle infection (Table 3.2). There was, however, a significant SMLH by age interaction effect on strongyle intensity (Table 3.2). The correlation between SMLH and strongyle intensity differed for animals of different ages, with the strongest effects apparent in older individuals than in younger individuals. There was also a marginal, but non-significant, SMLH by capture year interaction ($P = 0.07$).

For lungworms, older gazelles and gazelles caught in 2009 had higher parasite intensities than younger gazelles and those caught in 2011 (Table 3.2). SMLH also had a significant main effect on lungworm intensity, with intensity decreasing with increasing heterozygosity (Table 3.2). There was also a significant SMLH by capture year interaction (Table 3.2), where lungworm intensity declined with SMLH for individuals caught in 2009 but not for individuals caught in 2011 (Fig. 3.1b). No factor was significantly associated with parasite richness, but age had a marginal positive effect ($P = 0.054$).

B. Longitudinal analysis

For the 25 gazelles sampled over a 12 month period (July 2011-June 2012), all individuals were infected with strongyle nematodes and lungworms, and intensity ranged from 50-5,750 EPG (mean = 1500) and from 1-4,788 LPG (mean = 409.9) respectively. Parasite richness ranged from 2 to 6 taxa, with an average of 4.1. Strongyle intensity was negatively correlated with age and SMLH (Table 3.3). In addition, we found a significant SMLH by age interaction effect (Table 3.3). The correlation between SMLH and strongyle intensity differed for individuals of different ages, with stronger effects observed in younger individuals than in older individuals.

There was no main effect of SMLH or season on lungworm intensity (Table 3.3). However, there was a marginal SMLH by season interaction ($P = 0.052$), where the negative correlation between lungworm intensity and SMLH was stronger during the dry season than the wet season. Parasite richness varied significantly by sex (males > females) and season (wet > dry) (Table 3.3).

DISCUSSION

Heterozygosity-fitness correlations are widely used to explore the effects of inbreeding in natural populations, however, across studies, observed patterns of association are highly inconsistent (e.g. Acevedo-Whitehouse et al. 2006; Rijks et al. 2008; Chapman et al. 2009; Fox & Reed 2011; Harrison et al. 2011). One primary hypothesis for these inconsistencies is that correlations are generally weak (reviewed in Chapman et al. 2009), but other possible reasons may be differences in the fitness-related traits examined; in individual characteristics; and in the environmental condition that individuals experience (Lens et al. 2000; Pujolar et al. 2006; Szulkin et al. 2007; Shaner et al. 2013; Annavi et al. 2014). In our study, we found evidence for context-dependent HFCs, where the strength of correlations between heterozygosity and parasite intensities depended on host age and environmental conditions. For strongyle nematodes, correlations between intensity and heterozygosity differed for individuals of different ages. For the cross-sectional analysis, we found that effects were stronger in older individuals than in younger individuals, and in the longitudinal analysis, effects were stronger in younger individuals than in older individuals. For lungworms, negative correlations between parasite intensity and heterozygosity were stronger in 2009 than in 2011, and during the dry season compared to the wet season. Importantly, these context-dependent HFCs were consistently observed at both a single time point and over the course of twelve months. Furthermore, because

we found significant variance in inbreeding as measured by identity disequilibrium, our estimates of heterozygosity and the observed HFCs may reflect genome-wide effects (Miller & Coltman 2014).

The associations between individual heterozygosity and parasite intensity we observed in the Grant's gazelles differed by parasite taxa. For strongyle nematodes, we found that HFCs were age-dependent, and this was consistently observed at both a single time point (at capture) and over the course of twelve months. However, the strength of these associations differed for individuals of different ages. In our cross-sectional analysis, we found that correlations between heterozygosity and strongyle intensity were stronger in older individuals than in younger individuals, and in our longitudinal analyses, effects were stronger in younger individuals than in older individuals. Theory predicts that HFCs are more likely to be detected in younger age classes because the variability in fitness components such as growth and survival differences are likely to be higher during early stages of development (Charlesworth & Hughes 1996; David 1998). For example, in Alpine marmots (*Marmota marmota*), significant positive associations between individual heterozygosity and survival were observed in juveniles but not adult individuals, likely because unfit individuals did not survive to adulthood (Cohas et al. 2009). On the other hand, HFCs may be less detectable in younger age classes because important selective pressures occurring early in life mask the fitness effects of inbreeding (Keller & Waller 2002). Age-dependent HFCs have also been observed for parasite infections. In harbor seals, lungworm prevalence (*Otostrongylus circumlitus* and *Parafilaroides gymnurus*) was negatively correlated with heterozygosity in juveniles, but not adults (Rijks et al. 2008); while in Soay sheep (*Ovis aries*), strongyle nematode egg counts were negatively correlated with heterozygosity in adults but not lambs (Coltman et al. 1999). In Grant's gazelles, we found that in some instances,

correlations between heterozygosity and strongyle intensity were stronger in older individuals, and in other instances, effects were stronger in younger individuals (i.e. cross-sectional vs. longitudinal analyses). This suggests that age-effects may be context-dependent, and that correlations are generally stronger for more vulnerable age groups.

For lungworm infections in Grant's gazelles, we found that environment was an important determinant of the strength of HFCs. Negative associations between lungworm intensity and heterozygosity were stronger in 2009 compared to 2011, as well as in the dry season compared to the wet season. In a recent meta-analysis, Fox & Reed (2011) found that inbreeding depression is typically stronger under stressful conditions such as when individuals are exposed to toxic chemicals, heat stress, or intraspecific competition. For example, in tadpoles (*Rana temporaria*), associations between offspring survival and heterozygosity were only observed when resources were limited (Lesbarreres et al. 2005), while inbreeding effects on developmental instability in Taita thrushes (*Turdus helleri*) were more pronounced under high levels of environmental disturbance (Lens et al. 2000). However, other studies have detected HFCs under more benign conditions. In badgers (*Meles meles*), for instance, cubs with more heterozygous fathers had higher rates of survival than cubs with less heterozygous fathers, but only during wet summers, presumably because moist soil conditions enhanced badger food supply (Annavi et al. 2014). In our study, the stronger heterozygosity-lungworm association observed in 2009 and in the dry season might reflect stronger inbreeding effects during low resource conditions. Our sampling year of 2009 was the driest year on record at our field site in the past 14 years (mean annual rainfall: 1999-2013: 649 mm; 2009: 324 mm) and received almost a third less rainfall than 2011 (rainfall: 1016.4 mm). Similarly, average monthly rainfall over the 12 month longitudinal study was 18 times greater during the wet season than during the

dry season. Periods of reduced rainfall in semi-arid savannahs strongly influence vegetation growth and quality, often reducing the amount of food available to ungulate species (Ogutu et al. 2008). Low precipitation in 2009 and during the dry season likely reduced the forage available to Grant's gazelles at our study site, which may have affected the nutritional condition of gazelles and mediated the observed HFCs.

Another interesting finding of our study is that the context that influenced HFC patterns varied for different parasite taxa. Other studies have shown that even in the same study system main effects of heterozygosity can be observed for some parasites and not others. For example, in ring-tailed lemurs (*Lemur catta*), HFCs were detected for ectoparasites such as larvae of the fly *Cuterebra* species, but not for gastrointestinal parasites such as strongyles, strongyloides and entamobea (Charpentier et al. 2008). Similarly, in raccoons (*Procyon lotor*), individuals with high levels of genetic diversity had fewer endoparasites, individuals with intermediate levels of genetic diversity had fewer replete ticks (*Dermacentor variabilis*), and genetic diversity did not predict raccoon infection with non-replete ticks (*Trichodectes octomaculatus*) (Ruiz-Lopez et al. 2012). In addition to differences in the main effect of heterozygosity on parasitism observed in other studies, we show that context-dependent effects also differ. Hence, the cost of infection may not only differ for different parasite taxa, but these effects may only be detectable for certain types of individuals and under certain conditions.

Understanding the circumstances under which individual genetic diversity effects fitness is a critical step towards understanding how inbreeding can negatively affect natural populations. Correlations between heterozygosity and fitness are often relatively weak, but several recent studies have shown that the magnitude and directions of these associations may vary with context (e.g. Acevedo-Whitehouse et al. 2006; Rijks et al. 2008; Chapman et al. 2009; Fox &

Reed 2011; Harrison et al. 2011). By examining HFCs at a single time point and over the course of twelve months, our work demonstrates that associations between heterozygosity and common parasite infections are consistently context-dependent, and the context differs for different parasite taxa. This highlights the cryptic ways by which low genetic diversity may negatively affect natural populations.

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REFEENCES

Acevedo-Whitehouse, K., Gulland, F., Greig, D., & Amos, W. (2003). Inbreeding: disease susceptibility in California sea lions. *Nature*, *422*(6927), 35-35.

Acevedo-Whitehouse, K. A. R. I. N. A., Spraker, T. R., Lyons, E., Melin, S. R., Gulland, F., Delong, R. L., & Amos, W. (2006). Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups.*Molecular Ecology*, *15*(7), 1973-1982.

Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D., & Coulson, T. (2001). The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London B: Biological Sciences*, *268* (1480), 2021-2027.

Annavi, G., Newman, C., Buesching, C. D., Macdonald, D. W., Burke, T., & Dugdale, H. L. (2014). Heterozygosity–fitness correlations in a wild mammal population: accounting for parental and environmental effects. *Ecology and evolution*, *4*(12), 2594-2609.

Aparicio, J. M., Ortego, J., & Cordero, P. J. (2006). What should we weigh to estimate heterozygosity, alleles or loci?. *Molecular Ecology*, *15*(14), 4659-4665.

Balloux, F., Amos, W., & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations?. *Molecular Ecology*, *13*(10), 3021-3031.

Bates D, Maechler M, Bolker B (2011). *lme4: Linear Mixed-Effects Models Using S4 Classes*. R package version 0.999375-38, URL http://CRAN.R-project.org/package=lme4.

Brommer, J. E., Kekkonen, J., & Wikström, M. (2014). Using heterozygosity–fitness correlations to study inbreeding depression in an isolated population of white-tailed deer founded by few individuals. *Ecology and Evolution*.

Cain, B., Wandera, A. B., Shawcross, S. G., Edwin Harris, W., Stevens-Wood, B. A. R. R. Y., Kemp, S. J. & Watts, P. C. (2014). Sex-Biased Inbreeding Effects on Reproductive Success and Home Range Size of the Critically Endangered Black Rhinoceros. *Conservation biology*, *28*(2), 594-603.

Chapman, J. R., Nakagawa, S., Coltman, D. W., Slate, J., & Sheldon, B. C. (2009). A quantitative review of heterozygosity–fitness correlations in animal populations. *Molecular Ecology*, *18*(13), 2746-2765.

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual review of ecology and systematics*, 237-268.

Charlesworth, B., & Hughes, K. A. (1996). Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence.*Proceedings of the National Academy of Sciences*, *93*(12), 6140-6145.

Charpentier, M. J., Williams, C. V., & Drea, C. M. (2008). Inbreeding depression in ring-tailed lemurs (Lemur catta): genetic diversity predicts parasitism, immunocompetence, and survivorship. *Conservation Genetics*, *9*(6), 1605-1615.

Crnokrak, P., & Roff, D. A. (1999). Inbreeding depression in the wild. *Heredity*,*83*(3), 260-270.

Cohas, A., Bonenfant, C., Kempenaers, B., & Allaine, D. (2009). Age-specific effect of heterozygosity on survival in alpine marmots, Marmota marmota.*Molecular ecology*, *18*(7), 1491-1503.

Coltman, D. W., Bowen, W. D., & Wright, J. M. (1998). Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1398), 803-809.

Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, 1259-1267.

Coltman, D. W., & Slate, J. (2003). Microsatellite measures of inbreeding: A meta-analysis. *Evolution*, *57*(5), 971-983.

Cote, S. D., Stien, A., Irvine, R. J., Dallas, J. F., Marshall, F., Halvorsen, O., ... & Albon, S. D. (2005). Resistance to abomasal nematodes and individual genetic variability in reindeer. *Molecular Ecology*, *14*(13), 4159-4168.

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J. & Clutton-Brock, T. H. (1998). Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London B: Biological Sciences*,*265*(1395), 489-495.

Crawley, M. J. (2014). *Statistics: an introduction using R*. John Wiley & Sons.

Crow, J. F. (1948). Alternative hypotheses of hybrid vigor. *Genetics*, *33*(5), 477.

David, P. (1998). Heterozygosity–fitness correlations: new perspectives on old problems. *Heredity*, *80*(5), 531-537.

David, P., Pujol, B., Viard, F., Castella, V., & Goudet, J. (2007). Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*,*16*(12), 2474-2487.

DeRose, M. A., & Roff, D. A. (1999). A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution*, 1288-1292.

DeWoody, A.J., Schupp, J., Kenefic, L., Busch, J., Murfitt, L., & Keim, P. (2004). Universal method for producing ROX-labeled size standards suitable for automated genotyping. BioTechniques 37:348-350.

East, R. (Ed.). (1999). *African antelope database 1998* (Vol. 21). IUCN.

Ezenwa, V. O. (2003). Habitat overlap and gastrointestinal parasitism in sympatric African bovids. *Parasitology, 126*(4), 379-388. doi: 10.1017/s0031182002002913

Ezenwa, V. O. (2004). Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology*, *15*(3), 446-454. doi: 10.1093/beheco/arh028

Ezenwa, V. O., Stefan Ekernas, L., & Creel, S. (2012). Unravelling complex associations between testosterone and parasite infection in the wild. *Functional Ecology, 26*(1), 123-133. doi: 10.1111/j.1365-2435.2011.01919.x

Forrester, S. G., & Lankester, M. W. (1997). Extracting protostrongylid nematode larvae from ungulate feces. *Journal of Wildlife Diseases, 33*(3), 511-516.

Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution, 65*(1), 246-258.

Frankham, R. (1995). Inbreeding and extinction: a threshold effect. *Conservation biology, 9*(4), 792-799.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biologies, 326*, 22-29.

Haanes, H., Markussen, S. S., Herfindal, I., Røed, K. H., Solberg, E. J., Heim, M.& Sæther, B. E. (2013). Effects of inbreeding on fitness-related traits in a small isolated moose population. *Ecology and evolution, 3*(12), 4230-4242.

Hansson, B., & Westerberg, L. (2008). Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects?. *Conservation Genetics, 9*(1), 73-83.

Harrison, X. A., Bearhop, S., Inger, R., Colhoun, K., Gudmundsson, G. A., Hodgson, D., & Tregenza, T. O. M. (2011). Heterozygosity–fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects. *Molecular ecology, 20*(22), 4786-4795.

Hawley, D. M., Sydenstricker, K. V., Kollias, G. V., & Dhondt, A. A. (2005). Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. *Biology Letters, 1*(3), 326-329.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology*, arq041.

Hurtrez-Boussès, S., Perret, P., Renaud, F., & Blondel, J. (1997). High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. *Oecologia*, *112*(4), 514-517.

IUCN (2008). The IUCN red list of threatened species. Available from: <www.redlist.org>.

Kardos, M., Allendorf, F. W., & Luikart, G. (2014). Evaluating the role of inbreeding depression in heterozygosity-fitness correlations: how useful are tests for identity disequilibrium?. *Molecular ecology resources*, *14*(3), 519-530.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230-241.

Kruuk, L. E., Sheldon, B. C., & Merilä, J. (2002). Severe inbreeding depression in collared flycatchers (Ficedula albicollis). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1500), 1581-1589.

Lande, R. (1988). Genetics and demography in biological conservation.*Science*, *241*(4872), 1455-1460.

Lesbarreres, D., Primmer, C. R., Laurila, A., & Merilae, J. (2005). Environmental and population dependency of genetic variability-fitness correlations in Rana temporaria. *Molecular Ecology*, *14*(1), 311-323.

Lens, L., Van Dongen, S., Galbusera, P., Schenck, T., Matthysen, E., & Van de Casteele, T. (2000). Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. *Journal of Evolutionary Biology*, *13*(6), 889-896.

Lorenzen, E. D., Arctander, P., & Siegismund, H. R. (2008). Three reciprocally monophyletic mtDNA lineages elucidate the taxonomic status of Grant's gazelles. *Conservation Genetics*, *9*(3), 593-601.

Lynch, M., Conery, J., & Burger, R. (1995). Mutation accumulation and the extinction of small populations. *American Naturalist*, 489-518.

MacDougall-Shackleton, E. A., Derryberry, E. P., Foufopoulos, J., Dobson, A. P., & Hahn, T. P. (2005). Parasite-mediated heterozygote advantage in an outbred songbird population. *Biology Letters*, *1*(1), 105-107.

Miller, J. M., & Coltman, D. W. (2014). Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta-analysis. *Molecular ecology*, *23*(8), 1899-1909.

Nieminen, M., Singer, M. C., Fortelius, W., Schöps, K., & Hanski, I. (2001). Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *The American Naturalist*, *157*(2), 237-244.

Ogutu, J. O., Piepho, H. P., Dublin, H. T., Bhola, N., & Reid, R. S. (2008). Rainfall influences on ungulate population abundance in the Mara-Serengeti ecosystem. *Journal of Animal Ecology*, *77*(4), 814-829.

Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular ecology notes*, *6*(1), 288-295.

Pujolar, J. M., Maes, G. E., Vancoillie, C., & Volckaert, F. A. M. (2006). Environmental stress and life-stage dependence on the detection of heterozygosity-fitness correlations in the European eel, Anguilla anguilla.*Genome*, *49*(11), 1428-1437.

Rijks, J. M., Hoffman, J. I., Kuiken, T., Osterhaus, A. D. M. E., & Amos, W. (2008). Heterozygosity and lungworm burden in harbour seals (Phoca vitulina).*Heredity*, *100*(6), 587-593.

Ruiz-López, M. J., Gañan, N., Godoy, J. A., Del Olmo, A., Garde, J., Espeso, G., ... & Gomendio, M. (2012). Heterozygosity-Fitness Correlations and Inbreeding Depression in Two Critically Endangered Mammals. *Conservation Biology*, *26*(6), 1121-1129.

Ryder, T. B., Tori, W. P., Blake, J. G., Loiselle, B. A., & Parker, P. G. (2010). Mate choice for genetic quality: a test of the heterozygosity and compatibility hypotheses in a lek-breeding bird. *Behavioral Ecology*, *21*(2), 203-210.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature*,*392*(6675), 491-494.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234.

Shaner, P. J. L., Chen, Y. R., Lin, J. W., Kolbe, J. J., & Lin, S. M. (2013). Sex-specific correlations of individual heterozygosity, parasite load, and scalation asymmetry in a sexually dichromatic lizard. *PloS one*, *8*(2), e56720.

Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M., & Clutton-Brock, T. H. (2000). Inbreeding depression influences lifetime breeding success in a wild population of red deer (Cervus elaphus). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1453), 1657-1662.

Slate, J., & Pemberton, J. M. (2002). Comparing molecular measures for detecting inbreeding depression. *Journal of Evolutionary Biology*, *15*(1), 20-31.

Sperry, J. H., Butler, L. K., Romero, L. M., & Weatherhead, P. J. (2009). Effects of parasitic infection and radio-transmitters on condition, hematological characteristics and corticosterone concentrations in Texas ratsnakes. *Journal of Zoology*, *278*(2), 100-107.

Szulkin, M., Garant, D., McCleery, R. H., & Sheldon, B. C. (2007). Inbreeding depression along a life-history continuum in the great tit. *Journal of evolutionary biology*, *20*(4), 1531-1543.

Szulkin, M., Bierne, N., & David, P. (2010). Heterozygosity-fitness correlations: a time for reappraisal. *Evolution*, *64*(5), 1202-1217.

Ujvari, B., & Madsen, T. (2006). Age, parasites, and condition affect humoral immune response in tropical pythons. *Behavioral Ecology*, *17*(1), 20-24.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535-538.

Velando, A., Barros, Á., & Moran, P. (2015). Heterozygosity–fitness correlations in a declining seabird population. *Molecular ecology*, *24*(5), 1007-1018.

Voegeli, B., Saladin, V., Wegmann, M., & Richner, H. (2012). Parasites as mediators of heterozygosity–fitness correlations in the Great Tit (Parus major).*Journal of evolutionary biology*, *25*(3), 584-590.

Von Hardenberg, A., Bassano, B., FESTA-BIANCHET, M. A. R. C. O., Luikart, G., Lanfranchi, P., & Coltman, D. (2007). Age-dependent genetic effects on a secondary sexual trait in male Alpine ibex, Capra ibex. *Molecular Ecology*,*16*(9), 1969-1980.

Westemeier, R. L., Brawn, J. D., Simpson, S. A., Esker, T. L., Jansen, R. W., Walk, J. W., ... & Paige, K. N. (1998). Tracking the long-term decline and recovery of an isolated population. *Science*, *282*(5394), 1695-1698.

Worsley-Tonks, K. E., Lance, S. L., Beasley, R. R., Jones, K. L., & Ezenwa, V. O. Development and characterization of 30 novel microsatellite markers for Grant's gazelle (Nanger granti). *Conservation Genetics Resources*, 1-3.

Table 3.1: Characteristics of the twelve microsatellite loci.
Na, number of alleles; **Ho**, observed heterozygosity; **He**, expected heterozygosity; **HWE**, Hardy-Weinberg equilibrium. The frequencies of null alleles were estimated using the Brookfield 1estimator in Micro-Checker. Loci depicted with an asterisk deviated from HWE.

Locus	N	Na	Ho	He	HWE	Null alleles
Nagr 12	102	13	0.775	0.84	0.09	0.0314
Nagr 13	102	4	0.235	0.227	1	0.0051
Nagr 20	103	8	0.476	0.501	0.26	0.0307
Nagr 23	101	6	0.624	0.675	0.77	0.0277
Nagr 27	103	15	0.883	0.876	0.6	-0.0086
Nagr 29*	102	10	0.784	0.813	0	0.0209
Nagr 32	102	5	0.627	0.662	0.04	0.0312
Nagr 33*	103	4	0.553	0.473	0	-0.0462
Nagr 35*	102	6	0.902	0.703	0	-0.1198
Nagr 36	103	5	0.583	0.561	0.14	-0.0095
Nagr 44	97	7	0.639	0.709	0.75	0.0395
Nagr 48	103	4	0.65	0.648	0.71	-0.0036

Table 3.2: Minimum adequate models showing main and interaction effects of SMLH (standard multilocus heterozygosity) on three measures of parasite infection (strongyle intensity, lungworm intensity, and parasite richness) in Grant's gazelles. Strongyle intensity and parasite richness analyses were run using general linear models (with *F* values reported) while the lungworm analysis was a generalized linear model with a negative binomial distribution (with *z* values reported).
$^{+}p< 0.1$, $*p< 0.05$, $**p< 0.01$, $p< ***0.001$

Response variable	*Predictor variable*	*Df*	*F(or z) value*
Strongyle intensity	SMLH	1, 93	0.89
	Age	1, 93	11.13 **
	Capture year	1,93	8.12 **
	SMLH*age	1,93	4.21 *
	SMLH*capture year	1,93	3.41 $^{+}$
Lungworm intensity	SMLH	1	-2.15 *
	Age	1	4.4 ***
	Capture year	1	-4.47 ***
	SMLH*capture year	1	2.19 *
Parasite richness	Age	1,93	3.78 $^{+}$

Table 3.3: Minimum adequate mixed-effect models showing main and interaction effects of SMLH (standard multilocus heterozygosity) on three measures of parasite infection (strongyle intensity, lungworm intensity, and parasite richness) in Grant's gazelles.
$^{+}p< 0.1$, $*p< 0.05$, $**p< 0.01$, $p< ***0.001$

Response variable	*Predictor variable*	*Df*	*F value*
Strongyle intensity	SMLH	1	7.94**
	Age	1	5.04*
	SMLH*age	1	7.47*
Lungworm intensity	SMLH	1	2.46
	Season	1	1.38
	SMLH*season	1	3.8^{+}
Parasite richness	Sex	1	4.61*
	Season	1	12.16***

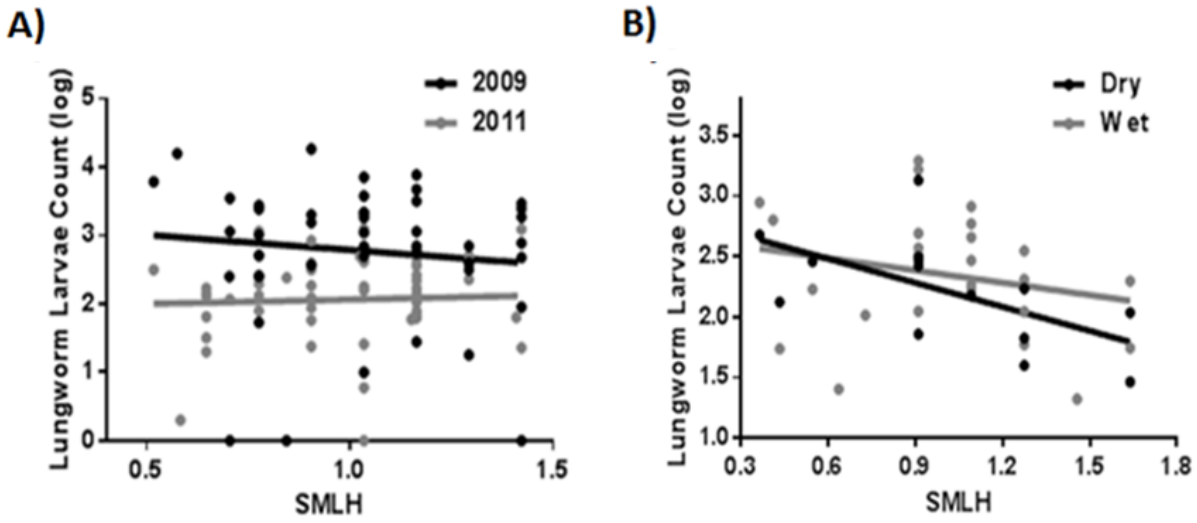

Figure 3.1: Relationship between standard multilocus heterozygosity (SMLH) and lungworm intensity for the **A)** cross-sectional analysis (black line: year 2009, grey line: year 2011) and **B)** longitudinal analysis (black line: dry season, grey line: wet season).

CHAPTER 4

CONCLUSIONS

Parasites can affect the fitness of their host in multiple ways, and macroparasites are notorious for negatively affecting host individuals in subtle and complex ways. Indeed, several studies have shown that parasites can affect host survival or reproduction by altering the behavioral response of individuals (Fox & Hudson 2001; Hanssen et al. 2003; Fenner & Bull 2008; Hillegass et al. 2010), affect certain individuals more than others (Gulland et al. 1993; Wilson et al. 2002; Rijks et al. 2008), and only under certain environmental conditions (Hudson et al. 1992; Coltman et al. 1999). This thesis explored complex mechanisms by which parasites can affect the fitness of their hosts. Chapter 2 examined how parasites influence time allocation to essential behaviors in female Grant's gazelles. Chapter 3 investigated when parasites are more likely to act against inbred individuals.

The purpose of Chapter 2 was to examine how experimental removal of parasitic worms influenced the relative amounts of time female Grant's gazelles allocated to core behaviors including foraging, vigilance, moving, and resting. The anthelmintic treatment effectively reduced female parasite load for ~120 days. During this timeframe, females relieved of their worms adjusted their daily time budgets and invested more time foraging at the expense of vigilance. In addition, group size also altered time investment to foraging versus vigilance behaviors. The results of this study illustrate that parasites can cause a major reallocation of behaviors, and these effects can be of similar magnitude to social drivers of behavioral time allocation.

The purpose of Chapter 3 was to examine associations between individual heterozygosity and parasite infection, and how these relationships varied with age, sex and environmental conditions (e.g. seasonality). Using cross-sectional and longitudinal approaches, this study revealed that associations between heterozygosity and parasite intensity varied consistently with context. For strongyle nematodes, correlations between intensity and heterozygosity differed for individuals of different ages, where in some instances associations were stronger in older individuals and in others instances correlations were stronger in younger individuals. For lungworms, relationships between heterozygosity and parasite intensity were stronger under low resource conditions. Hence, parasitism can be a consequence of low genetic diversity but only for certain individuals or under certain environmental conditions.

Taken together, this work illustrates that parasites can affect the fitness of host individuals in complex ways, often in conjunction with other social, ecological, and environmental factors. In Grant's gazelles, gastrointestinal and pulmonary nematode infections can alter how much time females invest in two fundamental behaviors: foraging and vigilance. As such, parasitic worm infections may indirectly affect the fitness of hosts by altering time investment to behaviors crucial for maintenance and survival. Moreover, individuals that are less genetically diverse may be more likely to suffer the consequences of infection, but only under certain conditions and at certain times. Overall, this thesis demonstrates that parasites can exert effects on hosts in subtle and complex ways that are manifested through host behavior, or on specific individuals, or at specific times.

REFERENCES

Coltman, D. W., Pilkington, J. G., Smith, J. A., & Pemberton, J. M. (1999). Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, 1259-1267.

Fenner, A. L., & Bull, C. M. (2008). The impact of nematode parasites on the behaviour of an Australian lizard, the gidgee skink Egernia stokesii. *Ecological Research, 23*(5), 897-903. doi: 10.1007/s11284-007-0453-1

Fox, A., & Hudson, P. (2001). Parasites reduce territorial behaviour in red grouse (Lagopus lagopus scoticus. In *Ecol. Lett.*

Gulland, F. M. D., Albon, S. D., Pemberton, J. M., Moorcroft, P. R., & Clutton-Brock, T. H. (1993). Parasite-associated polymorphism in a cyclic ungulate population. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *254*(1339), 7-13.

Hanssen, S. A., Folstad, I., Erikstad, K. E., & Oksanen, A. (2003). Costs of parasites in common eiders: effects of antiparasite treatment. *Oikos*, *100*(1), 105-111.

Hillegass, M. A., Waterman, J. M., & Roth, J. D. (2010). Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology*, arq041.

Hudson, P. J., Newborn, D., & Dobson, A. P. (1992). Regulation and stability of a free-living host-parasite system: Trichostrongylus tenuis in red grouse. I. Monitoring and parasite reduction experiments. *Journal of animal ecology*, 477-486.

Rijks, J. M., Hoffman, J. I., Kuiken, T., Osterhaus, A. D. M. E., & Amos, W. (2008). Heterozygosity and lungworm burden in harbour seals (Phoca vitulina).*Heredity*, *100*(6), 587-593.

Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Randolph, S. E., ... & Skorping, A. (2002). Heterogeneities in macroparasite infections: patterns and processes. *The ecology of wildlife diseases*, 6-44.

APPENDICES

DEVELOPMENT AND CHARACTERIZATION OF 30 NOVEL MICROSATELLITE MARKERS FOR GRANT'S GAZELLE (*NANGER GRANTI*)[3]

[3]Katherine E. L. Worsley-Tonks, Stacey L. Lance, Rochelle R. Beasley, Kenneth L. Jones, Vanessa O. Ezenwa (2015), *Conservation Genetic Resources,* 7, 219-221.

We isolated and characterized a set of 30novel microsatellite loci for Grant's gazelle (*Nanger granti*). Loci were screened in 24 individuals from a population in Laikipia County, Kenya. The mean number of alleles per locus was 3.73 (range1-10), and observed heterozygosity ranged from 0.00 to 0.870 (mean = 0.404).These new loci will facilitate basic behavioral, ecological, and population genetic studies of a species facing declining populations.

Grant's gazelle (*Nanger granti*) are widely distributed across East Africa from Ethiopia and South Sudan, across Kenya and into Uganda and central Tanzania (Kingdon & Hoffmann, 2013). Although general features of Grant's gazelle ecology and social organization have been described (Walther 1983), key characteristics of the species' biology remain unknown. In the face of downward trends in Grant's gazelle population numbers (IUCN 2008), insights drawn from microsatellite-based studies can contribute to a deeper understanding of gazelle behavior, ecology, and conservation.

We collected blood and ear punch samples from Grant's gazelles at the Mpala Research Centre, Laikipia, Kenya ($0^{o}17'$N, $37^{o}52'$ E) in 2009 and 2011. Blood samples were frozen at -20^{o}C and tissue samples were kept in 95% EtOH until DNA extraction. DNA extractions were performed using DNeasy blood and tissue kits (Qiagen) according the manufacturer's instructions. Total DNA extracted from the tissue of a single individual was used for isolation and identification of microsatellite loci following an Illumina shotgun sequencing protocol detailed in O'Bryhim et al. (2013). We tested forty-eight primer pairs for amplification and polymorphism (O'Bryhim et al. 2013), and assessed the variability of 30loci in 24 individuals (12 from 2009 and 12 from 2011).Conditions and characteristics of the loci are provided in Table 1. Tests for deviations from Hardy-Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). After Bonferroni correction, five loci

showed significant deviations from HWE. No linkage disequilibrium was detected. These new loci add to eight microsatellites described previously for *N. granti* (Huebinger et al. 2006).

Table 1: Details for 30 microsatellite loci developed for *Nanger granti*. The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is *N*; *k* is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for PCR.

Locus	**Primer Sequence 5' --> 3'**	**Repeat motif**	**Size (bp)**	**N**	***K***	**H_o**	**H_e**	**PI**	**TD**
Nagr2	F: * AGATTTCTGGAACTCTCTTGCC	TGC	240-264	24	3	0.125	0.119	0.78	TD 65
	R: AAATTGGCAACATCCACTGC								
Nagr5	F: *TTTAGCTTGAATTAAACCTGCTGC	AAAG	169-181	24	3	0.458	0.500	0.3	TD 65
	R: AATCCCATGAACGGAGATGC								
Nagr6	F: *GCAAATTCTTTACCGCTGGC	TGC	234-243	24	2	0.458	0.478	0.39	TD 65
	R: AATTTGACGGGCAAACAACC								
Nagr8	F: *TTTCTTTCAGTTCATTCATAGGGC	TGC	139-160	24	4	0.375	0.647	0.19	TD 65
	R: ACCTGGTGAGCTGCTATGGG								
Nagr12	F: *TCTTGTTTCTGTGCTTACATTGG	TGC	285-351	23	9	0.782	0.827	0.52	TD 65
	R: AGCTCTTCTGCTTTCCAGCC								
Nagr13	F: *AGTCGCATAGGGTCGGACG	TGC	248-266	24	3	0.292	0.254	0.58	TD 65
	R: ATTCTGGGTTCTGGAGACGC								
Nagr17	F: * CCACAGGACTATCCCATAAGGC	ATGG	208-212	23	2	0.217	0.364	0.47	TD 65
	R: CATTCACCTACTCAACCCACCC								
Nagr18†	F: *AAATTAGAATTTCTGTAGCTATGGCG	ATCT	245-265	24	5	0.375	0.753	0.10	TD 65
	R: CCCTCTATGATGAGGCACTCC								
Nagr20	F: *CTTCGAAAGAGTTCTCTCTGATGC	AATAG	200-210	24	3	0.375	0.362	0.46	TD 65
	R: CTGGAATTTCACACCCGTCC								

Nagr21	F: *TTGTTCTTCTATTTGGTATCCTATTGC	TTC	245-444	24	5	0.333	0.644	0.19	TD 65
	R: GAACAGAAACCTGTCCCTTGG								
Nagr22†	F: * CGACATCTTTGTTCTTCTGTAGTGG	TCTG	170-227	24	5	0.500	0.681	0.15	TD 65
	R: GAGACATGGCTTCTATCCCTGG								
Nagr23	F: *GGTTCAATGCCCTCCTCTGG	TTCC	259-283	24	4	0.458	0.609	0.22	TD 65
	R: GAGCCTGTCCCTACAGATCCC								
Nagr24	F: * CCCTCCTGAAATGTCCTCC	TCTG	237	24	1	0.000	0.000	0.10	TD 65
	R: GATATTTACTTTGGCACCCTTGG								
Nagr26	F: *CAACTTCCTATACAGATCCTCTGTTACC	ATGG	211-219	24	2	0.208	0.187	0.68	TD 65
	R: GCTCATTGTTTCTCCCATAAAGG								
Nagr27	F: *CACAGAAGACAGAGATGTTAAGTGC	TTC	207-261	23	10	0.870	0.846	0.40	TD 65
	R: GGAAACCCTTCTGCTTACTAGC								
Nagr28	F: * CCACCTGCCATGAAAGTTCC	ATGG	297	24	1	0.000	0.000	0.10	TD 65
	R: GGCTTTGGTAAGTATTGGGTGG								
Nagr29	F: *TCACCATGCTGCCTTAGACC	AAC	253-265	24	3	0.625	0.565	0.28	TD 65
	R: GGGAGCTTTGAAATCTAAAGAGG								
Nagr30	F: *AAGAAACAGGAGTTCAAATATGGG	AAGT	114-218	24	4	0.375	0.574	0.26	TD 65
	R: GGGTCCCAAAGAGTAGGGC								
Nagr31	F: * CTGCTGTTCCTGTGAGGGC	ATGG	206-214	24	2	0.375	0.353	0.48	TD 65
	R: GGTAGAGACATTTAGGGTTAGCTAGGG								
Nagr32	F: *TTAGACAGGCATCAATCTCTGC	ATC	193-203	24	4	0.333	0.640	0.19	TD 65
	R: GGTATAGAGCAAGCACTTAACTCG								
Nagr33	F: *CAGACTGAAGTCTTTCCCACCC	AAC	402-417	24	4	0.625	0.666	0.18	TD 65

	R: GGTCTCCCAGATAGCATCCC								
Nagr35	F: *GGTTTGCACAGAGTAGGACACG	TGC	233-263	23	4	0.565	0.575	0.24	TD 65
	R: GTGCTGTGATTTGTTGCTGC								
Nagr36	F: *TTCTTTCTTCCTATCTGCCTGC	ATCT	203-223	24	4	0.542	0.532	0.32	TD 65
	R: GTTTGGCCTCCAAATAGAGC								
Nagr38†	F: * CTTTATAGCAAGTCCACCGAAGG	TGC	271-305	24	4	0.125	0.666	0.18	TD 65
	R: TCCTAGGTTGCAGTCCCTGG								
Nagr40†	F: * AAGGGTATGTTGTGCCGC	TGC	255-276	23	4	0.261	0.598	0.23	TD 65
	R: TCTGCTGCTATTTGGATATTTAGAGG								
Nagr41	F: *CCACAAACAGTCAGGCACG	AAAG	220-244	24	4	0.833	0.727	0.12	TD 65
	R: TCTTAACTGTTACTGCCTTCATCTCC								
Nagr42	F: *TGGGAAGAGAGTGTGGATGC	ATGG	304-348	24	4	0.417	0.563	0.25	TD 65
	R: TCTTTGGTAGATGAAGAGATACATGG								
Nagr44	F: *TGCTATGTGTTTGACATTGTGC	ATGG	374-390	19	4	0.632	0.691	0.15	TD 65
	R: TGTTGAGAACTGGCTAATGACG								
Nagr45†	F: * TATGCATGCTCAAGGTTGCC	AAAC	309-313	24	2	0.000	0.330	0.5	TD 65
	R: TTCCAGGTCCTACCTCCTAAGC								
Nagr48	F: *GGATGTGACTGAAACCCTGG	ATGG	354-362	24	3	0.583	0.617	0.22	TD 65
	R: TTTCTCATGGATTGCCTCCC								

* indicates CAG tag (5'-CAGTCGGGCGTCATCA -3') label;

† indicates significant deviations from Hardy-Weinberg expectations after Bonferroni correction.