



Original Investigation

Fluctuating asymmetry and blood parameters in three endangered gazelle species

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ABSTRACT

There is currently no agreement about the suitability of fluctuating asymmetry (FA) as an estimate of individual quality. We investigated the relationship between FA and health, a proxy for individual quality, in captive populations of three endangered gazelle species: *Gazella cuvieri*, *G. dama*, and *G. dorcas*. FA indices including information from sexual (horns) and/or non-sexual ordinary traits were calculated for each individual. Health was assessed using 15 blood parameters, and inbreeding coefficient was also included in the analyses. In the three species, the FA index was significantly related to at least one blood parameter (platelets, mean platelet cell volume, albumin, and lactate dehydrogenase), with levels indicating unhealthy condition in more asymmetric individuals. The exception was the negative relationship between FA and aspartate aminotransferase in ordinary traits of *G. cuvieri* and *G. dama*. FA was related to different blood parameters in every species and type of trait. As a large number of diseases can cause abnormal blood parameter levels in humans, we assumed that this also might be the case in gazelles. In general, the results suggest that FA is related to health in the three gazelle species studied, and thus, we concluded that FA can provide useful information about individual quality.

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Introduction

Fluctuating asymmetry (FA) is defined as any random deviation from perfect symmetry in characters with bilateral symmetry (Van Valen 1962; Palmer and Strobeck 1986), and reflects the ability of an organism to cope with stress during development (Leary and Allendorf 1989; Parsons 1990). The reasoning behind this is simple, as the same genotype controls the development of the right and the left sides of these characters and any difference between sides is due to developmental errors. Many different factors, both genetic (e.g. genetic variation, hybridisation, mutation) and environmental (e.g. temperature, nutrition, parasites), have been shown to affect the control of development, and thus, FA (reviews in Møller and Swaddle 1997; Polak 2003).

FA has been proposed as an estimate of phenotypic quality, assuming that some genotypes are better than others in producing stable phenotypes under certain environmental conditions (e.g. Møller and Swaddle 1997). The need to assess individual quality is common currency in ecological studies, but a prerequisite for FA to be useful in this assessment would be the existence of what is called the individual asymmetry parameter (Leamy 1993). In other words, developmental instability in one or a few

traits should be representative of developmental instability in the whole organism. In general, there is little consistency in the ranking of individuals within populations for FA of different traits (review in Møller and Swaddle 1997), but the low repeatability of FA, even in traits with completely shared developmental control mechanisms, might be responsible for this little consistency (Gangestad and Thornhill 1999). Subsequent reviews (Polak et al. 2003) including data from a large number of animal and plant species suggest that there is indeed an organism-wide control of development, although severely affected by trade-offs with specific mechanisms in certain traits (e.g. sexual or locomotor traits). The key question, however, about the use of FA as an estimate of individual quality is whether FA, or developmental instability in general, reflects fitness or not. The literature shows very mixed results, and different reviews reach different conclusions (Leung and Forbes 1996; Møller 1997, 1999; Tracy et al. 2003).

One predictor of fitness of special interest is health, an individual characteristic that is related to survival and shows the well-functioning of the organism. The association between FA and health has been studied mainly with regard to parasitic diseases (review in Møller 1996). Health can be assessed directly from the presence/absence of parasites, and indirectly by estimating the immune function and assuming that individuals with a stronger immune response will more successfully resist infection. Both types of health assessment seem to be related to FA (review in Møller

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2006). On the one hand, there is some evidence that the prevalence and intensity of parasitisation is related to more developmental instability (reviews in Møller, 1996, 2006). On the other hand, some studies suggest that FA reflects immunocompetence (e.g. Rantala et al., 2000, 2004). But not all diseases are caused by parasites. That is why we were interested in parameters providing information as broad as possible which could be related to any kind of illness. As suggested by ordinary health check-ups in people, blood analysis is one of the simplest but most informative methods of detecting health problems, and blood parameters can also be used as health indicators in other vertebrates (e.g. Rea et al. 1998; Newman et al. 2000; Milner et al. 2003). Modern devices, specially designed for medical science, enable us to assess a wide range of parameters from small amounts of blood, and every parameter provides information on a different aspect of health, such as infections, diet deficiencies or organ functioning.

In order to test the hypothesis that developmental instability predicts individual variation in phenotypic quality, we studied asymmetry and health in captive populations of *Gazella cuvieri*, *G. dama mhorri*, and *G. dorcas neglecta*, and predicted that FA would be related to some blood parameters. These three species are originally from North Africa, but all individuals studied here had been born and lived all their lives in captivity, and were involved in an ex-situ breeding programme. Any individual with high FA has probably experienced deficient developmental control, but a relationship between FA and health (blood parameters in our case) would imply that deficient developmental control has consequences for fitness. This is important, since any phenotypic variation not having consequences for fitness would be of little evolutionary or ecological interest. Obviously, a connection between FA and health does not mean that FA itself causes any disease. It could simply mean that an organism, under certain environmental and genetic conditions, experiences deficiencies in both developmental control and control of other functions leading to illness.

Most studies investigating FA in ungulates have focused on antler or horn asymmetry. Although some of these studies have failed to find any association between FA and fitness (e.g. Pélabon and Joly 2000), most of them show a significant negative relationship between FA and surrogates of fitness such as body size, dominance or reproductive success (Møller et al. 1996; Putman et al. 2000; Côté and Festa-Bianchet 2001; Mateos et al. 2008). Antler FA has been found to be related to both parasitisation (Folstad et al. 1996) and immunity (Lagesen and Folstad 1998) in the reindeer (*Rangifer tarandus*). FA has also been studied in the three *Gazella* species included in this study (in fact in the same captive populations), especially as related to inbreeding. Skull FA was positively related to inbreeding in *G. dama* and *G. dorcas* (Alados et al. 1995), while horn FA was positively related to inbreeding in *G. cuvieri* and *G. dama* (Roldan et al. 1998; Gomendio et al. 2000). Interestingly, in *G. cuvieri*, inbreeding was related to sperm quality, and sperm quality to horns FA (Roldan et al. 1998; Gomendio et al. 2000). Thus, FA in this species can be considered an indicator of genetic stress, and, indirectly, of male reproductive success. The effect of inbreeding on fitness has been shown to be important in these captive populations, since inbreeding was related to both parasitisation (Cassinello et al. 2001) and longevity (Cassinello 2005). However, the possibility of a direct relationship between FA and health has not been previously explored in these species.

Material and methods

The species subject of study

The three species studied were the Cuvier's gazelle (*Gazella cuvieri* Ogilby 1841), the mhorri gazelle (*G. dama mhorri* Ben-

net 1833) and the dorcas gazelle (*G. dorcas neglecta* Lavauden 1926) (family Bovidae, subfamily Antilopinae). All three are North African gazelles, *G. cuvieri* living mainly in mountainous regions, and the other two species typically in the plains. Although the three species face high risk of extinction in the wild, they are threatened to differing degrees. As a species, according to IUCN (2010), *G. dorcas* is classified as vulnerable, *G. cuvieri* as endangered, and *G. dama* as critically endangered. The subspecies *G. dama mhorri* has almost certainly been extinct in the wild since 1968, although a few individuals have been reintroduced in some African countries (Wiesner and Müller 1998). All the individuals included in the present study live in captivity in the Parque de Rescate de la Fauna Sahariana (PRFS) in Almería (south-eastern Spain), a facility belonging to the Consejo Superior de Investigaciones Científicas (Spanish National Research Council). The PRFS accommodates around one hundred individuals of each species, all descended from a few individuals brought to Almería from the Western Sahara in the 1970s. The breeding programme carried out in the PRFS for almost 40 years has succeeded in increasing the size of the captive populations, establishing new reproductive groups at other institutions, and providing animals for reintroduction in North Africa, for example *G. dorcas* in Senegal, or *G. cuvieri* and *G. dama* in Tunisia. Most animals in the PRFS live in reproductive herds (5–12 individuals) in relatively small (400–1500 m²) fenced enclosures, although some adult males are kept in smaller (20–80 m²) individual pens. Their diet consists of commercial pellets, fresh alfalfa hay and barley grain. Water and mineral salts are available ad libitum. All animals are subject to strict veterinary control. Inbreeding coefficient (IC) for every individual was calculated from studbook data using the software SPARKS v. 1.54 (<http://www.isis.org/CmsHome/content/SPARKS>).

Morphological measurements and blood sampling

All individuals included in this study were caught in October 2001 for routine vaccination, and we took advantage of the occasion to take morphological measurements and blood samples. The animals were captured with nets, immobilized by tying their legs together with a rope, and their eyes were covered with a cloth to tranquilise them. A 5-ml blood sample was taken from the jugular vein with a syringe and the animal was immediately vaccinated subcutaneously. When all animals caught on a particular day had been vaccinated, we proceeded with measurement. Forelegs were measured from the tarsal to the fetlock joint after flexing the knee and bending the hoof backwards. This part of the leg corresponds mostly to the metacarpal bone, so hereafter it is called the metacarpus. Similarly, the hind legs were measured from the hock to the fetlock joint after flexing the hock and bending the hoof backwards. As this part of the leg corresponds mostly to the metatarsal bone, hereafter it is called the metatarsus. Horn width was measured at the base of every horn in two different ways: greatest lateromedial width and greatest oral–aboral width. Horn length was not measured because of the possibility of its being very worn down. In the case of *G. cuvieri*, horns were completely sheathed in rubber tubes to prevent injury, making horn width measurements difficult and unreliable. Any individual with a missing horn was not included in the study. Finally, ear length was also measured from the notch between the tragus and antitragus to the tip. Legs and horns were measured with a digital calliper to the nearest 0.01 mm, and ear length with a ruler to the nearest 0.1 cm. All traits were measured twice to estimate repeatability and by the same person (M.D.) to avoid inter-observer variability. The second set of measurements was taken when all first measurements for a particular individual were completed.

Blood analyses

After extraction, blood samples were refrigerated for 2–5 hours before analysis. Around 0.5 ml of blood was used to estimate blood cell parameters using an electronic cell counter (Cellanalyzer CA530 Oden, Medonic, Stockholm, Sweden). Blood cell parameters included in this study were red blood cells (RBC), mean red blood cell volume (MCV), haematocrit (HTC), red blood cell distribution width (RDW), platelets (PLT), mean platelet cell volume (MPV), white blood cells (WBC), and haemoglobin concentration (HGB). This cell counter was designed for human blood analysis, and maximums and minimums provided for some parameters are not appropriate for gazelle blood. Therefore, mean cell haemoglobin and mean cell haemoglobin concentration could not be estimated. Although certain cell counter discriminator levels had been adapted to the characteristics of gazelle blood (for example the threshold size discriminating platelets and red blood cells), classification of white blood cells by size following human threshold sizes does not make sense in gazelles, and therefore information for small, middle-sized and large white blood cells has not been included. The rest of the blood was centrifuged (5000 r.p.m. for 15 min) and the plasma separated for biochemical analyses using a dry-chemistry blood analyser (Spotchem SP 4410, Menarini Diagnostics, Florence, Italy) with Spotchem II reagent strips, panels 1 and 2 (Arkray Factory, Kyoto, Japan). The parameters included in this study were glucose (GLU), total cholesterol (CHO), blood urea nitrogen (BUN), aspartate aminotransferase (GOT, formerly called glutamic-oxaloacetic transaminase), total proteins (PRO), albumin (ALB), and lactate dehydrogenase (LDH). As with the cell counter, the dry-chemistry blood analyzer was designed for human blood and some gazelle parameters were outside the range of the analyzer and could not be estimated. This was the case for total bilirubin, alanine aminotransferase, calcium, triglycerides, and uric acid.

Analysis of fluctuating asymmetry

Since every morphological character was measured on the right (R) and left (L) sides of the body, it was possible to assess the asymmetry of these characters as $|R-L|$ (Palmer and Strobeck 2003). For *G. dama* ($n=30$ individuals) and *G. dorcas* ($n=55$) we had five morphological characters (metacarpus length, metatarsus length, maximum oral–aboral horn width, maximum latero–medial horn width, and ear length), and for *G. cuvieri* ($n=35$) only three (metacarpus length, metatarsus length, and ear length). Prior to the use of asymmetry values, a number of tests had to be done (Palmer and Strobeck 2003). First, we checked that R–L distributions did not differ significantly from a normal distribution (Kolmogorov–Smirnov test, $P \geq 0.10$ in all 13 cases). Second, we separated directional asymmetry and measurement error from other types of asymmetry (FA and antisymmetry) using a two-way ANOVA with individual and side as factors. In all 13 cases the variation explained by non-directional asymmetries (interaction individual \times side) was highly significant ($P < 0.0001$). Third, we checked that repeatability (Lessells and Boag 1987) of R–L was significant (range 0.454–0.883; $P < 0.001$ in all 13 cases), i.e., that R–L was significantly greater than measurement error. Fourth, we showed that repeatability of FA was also significant (range 0.288–0.862; $P \leq 0.018$ in all 13 cases). Fifth, and last, we checked whether FA was associated with the size of the trait by regressing the standardized FA on the standardized trait value. Standardization consisted of subtracting the mean from the value and dividing by the standard deviation (SD), thus giving a sample with mean = 0 and SD = 1. For most traits the regression was far from significant ($P > 0.10$), but showed a positive trend in *G. dama* for lateromedial horn width ($P = 0.038$), and a negative trend in *G. dorcas* for ear length ($P = 0.035$). However, none of these regressions was sta-

tistically significant after sequential Bonferroni-adjustment (Rice 1989), not even when applying the less conservative 10% level of significance (Chandler 1995). Therefore, there was no need to correct FA for trait size and absolute FA values were used.

Any relationship between FA and blood parameters could have been analyzed separately for each trait, but this approach is usually less powerful for this than the use of a composite statistic including FA from different traits, especially when FA from these traits has been standardized (Leung et al. 2000). Thus, a FA index, including information from all measured traits (five traits in *G. dama* and *G. dorcas*, and three traits in *G. cuvieri*), was calculated for each individual, as some researchers investigating FA do routinely (e.g. Zakharov et al. 2001; Brown et al. 2008). First, to assess this individual FA index, FA for every trait in each species was standardized (see standardization method above), and then the average of all the standardized FA for each individual was calculated. FA from every character thereby contributed equally to the FA index. Horns are considered sexual ornaments in males of these species (Gomendio et al. 2000) and FA of sexual and non-sexual (ordinary) traits may differ (Cuervo and Møller 1999). Consequently, FA indices for secondary sexual (maximum lateromedial and oral–aboral horn width) and ordinary traits (metacarpus, metatarsus and ear length) were also calculated separately in the two species with available horn measurements (*G. dama* and *G. dorcas*). The distribution of the FA index did not differ significantly from a normal distribution in any species, either considering all traits, only sexual traits or only ordinary traits (Kolmogorov–Smirnov test, $P \geq 0.15$ in the seven cases).

Statistical analyses

Before further analyses, all variables were checked for normality. GOT and LDH in *G. cuvieri*, GOT in *G. dama*, and BUN in *G. dorcas* were normalized by \log_{10} transformations, and IC in *G. dorcas* was normalized by square-root transformation. The other variables followed a normal distribution with no transformation (Kolmogorov–Smirnov test, $P \geq 0.10$). Since the three blood parameters reflecting blood oxygen carrying capacity (RBC, HTC and HGB) were strongly correlated with one another in the three species (Pearson correlation, $r \geq 0.568$, $30 < n < 55$, $P < 0.001$ in the nine tests), we proceeded to a Principal Components Analysis (PCA) and used the first factor resulting from this PCA in subsequent analyses. We first determined the best-fit model of blood parameters explaining variation in the FA index by using Akaike's information criterion (AIC; Burnham and Anderson 2002). The model with the lowest AIC (corrected for sample size, see Burnham and Anderson 2002) was considered the most parsimonious and plausible. IC was included in the analyses because of the known relationship between FA and inbreeding in these gazelle populations (see Introduction). Sex was also included as a factor. The sex distribution was six males and 29 females in *G. cuvieri*, 12 males and 18 females in *G. dama*, and 11 males and 44 females in *G. dorcas*. Sexual differences in the relationship between FA and blood parameters were not expected and, consequently, interaction terms were not included in the analyses. However, the possible effect of inbreeding on FA, particularly on FA of sexual ornaments (i.e., horns), might be different in males and females (horns are probably not sexual ornaments in females), and the interaction between sex and IC was thus included in the analyses. Once the best (most parsimonious) model explaining variation in the FA index in each species and type of trait (sexual and ordinary traits) had been determined, General Linear Models (GLM) were performed, including only the variables in the best model. All statistical analyses were two-tailed with a significance level of 0.05, and performed with the Statistica (StatSoft 2007) programme.

Table 1

General Mixed Models including only variables from best models (determined by using AIC, see the text) as independent variables and FA indices (including either all traits, only sexual traits or only ordinary non-sexual traits) as dependent variables in three gazelle species.

Species	Type of trait	Independent variables	Sum of squares	df	F	P
<i>G. cuvieri</i>	Ordinary traits	Sex	2.16	1	6.87	0.014
		GOT	3.18	1	10.10	0.0034
		PRO	1.21	1	3.84	0.060
		ALB	2.11	1	6.71	0.015
		Error	9.45	30		
<i>G. dama</i>	All traits	PLT	0.39	1	4.51	0.044
		MPV	1.28	1	14.96	<0.001
		BUN	0.24	1	2.84	0.10
		LDH	0.31	1	3.64	0.068
		Error	2.14	25		
	Ordinary traits	GOT	1.22	1	5.83	0.023
		LDH	1.42	1	6.80	0.015
		Error	5.65	27		
	Sexual traits	Sex	1.90	1	4.66	0.040
		MPV	2.38	1	5.83	0.023
		Error	10.99	27		
<i>G. dorcas</i>	All traits	LDH	1.16	1	5.39	0.024
		IC	0.50	1	2.32	0.13
		Error	11.17	52		
	Ordinary traits	LDH	0.87	1	2.21	0.14
		Error	20.73	53		
		Sex	0.44	1	0.94	0.34
	Sexual traits	BUN	1.11	1	2.37	0.13
		IC	4.01	1	8.53	0.0052
		Sex × IC	1.95	1	4.14	0.047
		Error	23.53	50		

Full models had the statistics: *G. cuvieri*, ordinary traits, $F_{4,30} = 2.82$, $r^2 = 0.273$, $P = 0.043$; *G. dama*, all traits, $F_{4,25} = 5.27$, $r^2 = 0.457$, $P = 0.0032$; *G. dama*, ordinary traits, $F_{2,27} = 4.72$, $r^2 = 0.259$, $P = 0.017$; *G. dama*, sexual traits, $F_{2,27} = 4.38$, $r^2 = 0.245$, $P = 0.022$; *G. dorcas*, all traits, $F_{2,52} = 3.41$, $r^2 = 0.116$, $P = 0.041$; *G. dorcas*, ordinary traits, $F_{1,53} = 2.21$, $r^2 = 0.040$, $P = 0.14$; *G. dorcas*, sexual traits, $F_{4,50} = 3.62$, $r^2 = 0.224$, $P = 0.011$.

Results

Best models explaining FA index variation included the following variables: sex, GOT, PRO and ALB (*G. cuvieri*, ordinary traits); PLT, MPV, BUN and LDH (*G. dama*, all traits); GOT and LDH (*G. dama*, ordinary traits); sex and MPV (*G. dama*, sexual traits); LDH and IC (*G. dorcas*, all traits); LDH (*G. dorcas*, ordinary traits); sex, BUN, IC and the interaction between sex and IC (*G. dorcas*, sexual traits) (Table 1).

In *G. cuvieri*, the FA index was significantly negatively related to GOT and ALB (Table 1, Fig. 1), and was larger in males than in females (Table 1; males, least squares (LS) mean ± SE = 0.74 ± 0.30 , $n = 6$, females, LS mean ± SE = -0.15 ± 0.11 , $n = 29$). In *G. dama*, the FA index including all traits was negatively related to PLT and positively related to MPV (Table 1, Fig. 2). However, when only ordinary traits were included, the FA index was related to different blood parameters, namely negatively related to GOT and positively related to LDH (Table 1, Fig. 2). When only sexual traits were considered, the FA index was significantly positively related to MPV (Table 1, Fig. 2) and was larger in males than in females (Table 1; males, least squares mean ± SE = 0.31 ± 0.19 , $n = 12$, females, LS mean ± SE = -0.21 ± 0.15 , $n = 18$). Finally, in *G. dorcas*, the relationship between the FA index and LDH was positive and significant when all traits were included (Table 1, Fig. 3), but was not significant when only ordinary traits were considered (Table 1). In this species, the FA index was significantly positively related to IC, but this relationship was more pronounced in males than in females (Table 1, Fig. 3).

Discussion

This study shows significant relationships between FA and blood parameters in three gazelle species, although these parameters are often different in each of those three species (ALB and GOT in *G.*

cuvieri; PLT, LDH, MPV and GOT in *G. dama*; LDH in *G. dorcas*). The particular blood parameters related to FA also differed depending on the type of morphological trait (ALB, LDH and GOT in non-sexual ordinary traits; MPV in sexual ornaments; PLT, MPV and LDH when both types of traits were considered). In general, the significant relationships between FA and blood parameters were as expected, i.e. higher levels of FA when blood parameter values indicated unhealthy conditions, with just one exception, the negative relationship between FA and GOT (see below). Therefore, the results suggest that FA is related to health in these species and, hence, there are grounds for considering an association between developmental instability and individual phenotypic quality.

It was not unexpected for FA to be related to different blood parameters in every species, because, even though all the animals live under similar environmental conditions in captivity, the three species differ in many aspects, e.g. body size, inbreeding coefficient, parasite load, etc. (Cassinello et al. 2001; Cassinello 2005). Thus, each might experience different health problems that would affect different blood parameters, mainly by increasing parameter range and variability. If FA is related to health, an increase in the variability of a blood parameter would make it easier to find significant relationships between FA and that particular parameter. Another non-exclusive explanation for the inconsistency of results in the three species would be that the studied populations are rather small and have undergone recent bottlenecks (Cassinello 2005). This usually implies a dramatic decrease in genetic variation and random fixation of characters, probably different ones in each species.

Basic information on blood parameters is available for different gazelle species (e.g. Dreverno et al. 1974; Rietkerk et al. 1994; Yarahoglu et al. 2004; Hammer et al. 2006; Konaş Aşkar et al. 2007), including the three species studied here (Bush et al. 1981; Peinado et al. 1990; Abáigar 1993). However, to our knowledge, there is no study addressing the relationship between blood parameters and health condition in any member of the genus *Gazella*. There-

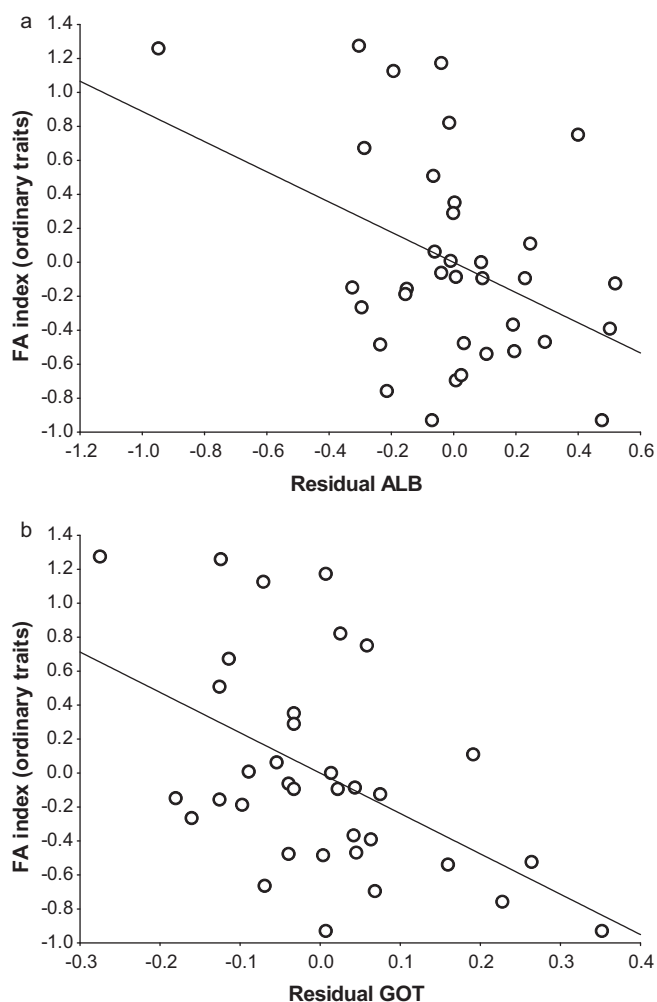


Fig. 1. FA index of non-sexual ordinary traits in relation to (a) residual ALB (residuals from regressing ALB on GOT and PRO, and including the sex as a fixed factor in the model) and (b) residual GOT (residuals from regressing GOT on ALB and PRO, and including the sex as a fixed factor in the model) in *G. cuvieri*. Lines represent the linear fits.

fore, we were forced to rely on literature from other mammals to explain the possible relationships between blood parameters and health in our three gazelle species. References from other Bovidae were used when available, but literature from non-bovid mammals, particularly humans, helped to complete the information.

Regarding blood cell parameters, a negative relationship between the FA index and PLT was found in *G. dama* when all traits were included in the FA index (Fig. 2a). PLT are cells in the blood that assist clotting, and low PLT levels (thrombocytopenia) thus increase the risk of haemorrhage. Low PLT levels can be caused by a decrease in PLT production or an increased rate of removal from blood, and in domestic cattle are associated with a large number of infectious (e.g. Omer et al. 2002; Wood et al. 2004; Bani Ismail & Dickinson 2010) and non-infectious (Pardío et al. 2007; Sobiech et al. 2008) diseases. Low PLT levels have also been found to be associated with cerebral ischemia in sheep (Littleton-Kearney et al. 1998) and viral infections (Scaradavou 2002) or leukaemia (Song et al. 1999) in humans. A positive relationship between MPV and FA index was found in *G. dama* when all traits or only sexual traits were used to calculate the FA index (Table 1, Fig. 2d). MPV indicates the size of PLT, and high MPV levels are associated with infectious diseases in cattle (Keller et al. 2006) and other mammal species (e.g. Lilliehöök et al. 1998; Yang et al. 2006; Thuita et al. 2008), and with an increased risk of stroke in humans (Bath et al. 2004). When low

PLT levels and high MPV levels happen at the same time, thrombocytopenia is probably due to increased destruction of platelets (Bessman et al. 1982).

Regarding blood chemistry parameters, a negative relationship between the FA index and ALB was found in *G. cuvieri* (Fig. 1a). ALB, which is synthesized in the liver, is the most abundant plasma protein in mammals, and serves a multitude of functions (e.g., hormone transport, pH control, maintenance of osmotic pressure in the circulatory system). Low plasma levels of this protein (hypoalbuminemia) have been found to be associated with parasitism in wild (Pérez et al. 2006) and domestic (Rowlands and Clampitt 1979; O'Kelly 1980) Bovidae, and with deficient nutrition in many Cervidae species (e.g. Wolkers et al. 1994; Sams et al. 1998; Säkkinen et al. 2005). In humans, low ALB levels are associated with a large number of diseases (review in Peters 1995). The main causes of low ALB are decreased synthesis due to malnutrition or liver disease and increased secretion due to nephrotic syndrome. High ALB can be caused by dehydration, but is usually interpreted as a symptom of good health. In addition, the FA index of ordinary traits in *G. dama* and all traits in *G. dorcas* was positively related to LDH (Figs. 2b and 3a). LDH is the enzyme that catalyzes pyruvate-lactate interconversion, and is used in veterinary and human medicine as an indicator of tissue damage, because LDH is abundant in the cells. Thus, high LDH levels in plasma have been associated, for example, with mastitis (Atroshi et al. 1996), and fascioliasis (Anderson et al. 1977) in cattle, and with heart attack (Chapelle et al. 1980), and cancer (Buccheri and Ferrigno 1994) in humans.

GOT is an enzyme present in cells of the liver, heart, skeletal muscle, kidney and other organs. GOT levels in blood plasma are low in healthy individuals, but when one of these organs is diseased or damaged, GOT is released into the bloodstream. High GOT levels have been found to be associated, for example, with toxin-induced muscle damage in sheep (Dailey et al. 2008), traumatic reticuloperitonitis in cattle (Gökçe et al. 2007), or fascioliasis in different domestic Bovidae (e.g. Wyckoff and Bradley 1985; Ferre et al. 1994; Yang et al. 1998). Contrary to our expectations, a negative relationship between FA index of ordinary traits and GOT was found in *G. cuvieri* and *G. dama* (Figs. 1b and 2c). We can briefly speculate about two possible explanations for this finding. First, some drugs used for infection treatment (e.g. metronidazole) may interfere with the plasma assay and cause factitiously low GOT (Rissing et al. 1978). If ill individuals (presumably those with high FA) had been treated for infections and the treatment caused low GOT, a negative relationship between FA and GOT could be found. However, we are not aware that any gazelle included in the study had received medication during at least one month before blood sampling. Second, it has been suggested that capture, handling and transportation of gazelles and other ungulates may affect a number of blood chemistry parameters, including GOT (e.g. Bush et al. 1981; English and Lephherd 1981; Vassart et al. 1994). If individuals in good condition (presumably those with low FA) struggled longer or with more intensity than individuals in poor condition and the struggle caused skeletal muscle damage and thus release of GOT into the blood, a negative relationship between FA and GOT could be found. Unfortunately, duration or intensity of struggle was not quantified. A negative relationship between a health-related parameter (blood mercury level) and GOT has also been found in a study of wild sea turtles captured in trawl nets (Day et al. 2007).

We do not know why the parameters related to FA were ALB, LDH or PLT and not others. Maybe it is not by chance that all these parameters show very little specificity, i.e., they are affected by many different illnesses. Although it would be difficult to reach a precise diagnosis by examining only these blood parameters, they are sensitive to a large number of diseases, and are therefore suitable for assessing the general health of an individual. For instance, high ALB levels are associated with a wide range of parasites and

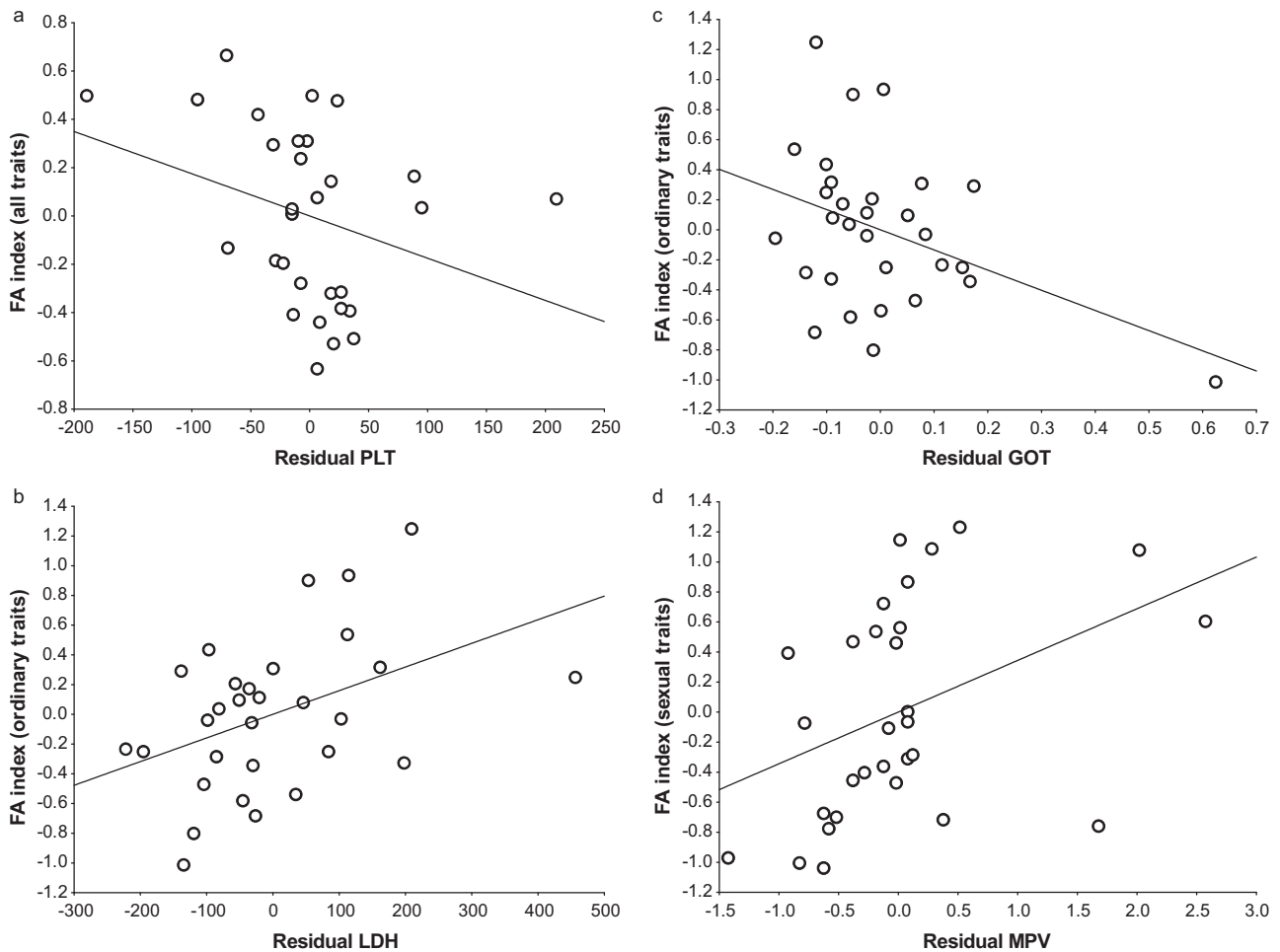


Fig. 2. FA index of (a) all morphological traits in relation to residual PLT (residuals from regressing PLT on MPV, BUN and LDH), (b) non-sexual ordinary traits in relation to residual LDH (residuals from regressing LDH on GOT), (c) non-sexual ordinary traits in relation to residual GOT (residuals from regressing GOT on LDH), and (d) sexual traits in relation to residual MPV (residuals after controlling for sexual differences) in *G. dama*. Lines represent the linear fits.

nutrition deficiencies (probably the two main causes of disease) in many ungulate species (see references above). Likewise, it has repeatedly been shown that ALB level is a good predictor of mortality risk in humans regardless of the cause of death (Goldwasser and Feldman 1997). The variety of diseases associated with high LDH or with the number and size of PLT in Bovidae and other mammals (see references above) would point in the same direction.

It is important to the interpretation of the results of this study to emphasize that all the animals studied here were born and had lived all their lives in captivity in the PRFS under very favourable conditions, with a balanced diet, specialized veterinary care, and absence of predators. Under these conditions, we would expect most individuals to be healthy with only slight variability in health-related traits such as blood parameters. Thus, significant relationships between FA and blood parameters would be detectable only if they were extremely strong, which makes the findings of this study still more relevant. Unfortunately, there is no information about blood parameters of these species in the wild, but more variable health conditions would be expected compared to captivity, because some animals might undergo food limitations and/or severe parasite infections. Although some unhealthy or diseased individuals might be eliminated from the population by predators or parasites, this would not compensate for the variable conditions the animals have to face in the wild. In addition, the studied populations have probably experienced a dramatic decrease in genetic variation (see above), which may also have resulted in a decrease in phenotypic

variation. If this is the case, these captive populations would show lower variability in anatomical and physiological traits than their wild relatives. Therefore, we predict that, in general, the relationship between FA and health will be stronger and easier to find in wild than in captive populations.

FA only was significantly related to inbreeding in *G. dorcas*, and only when sexual traits (horn measures) had been used to estimate the FA index (Table 1, Fig. 3b). As expected, the relationship was positive, i.e. higher FA in more inbred individuals, and more pronounced in males than in females, probably because horns only function as sexual traits in males. These results partially support previous studies in the same gazelle populations showing positive relationships between FA and inbreeding (see Introduction). Sexual differences in FA were statistically significant only in two cases, ordinary traits in *G. cuvieri* and sexual traits in *G. dama*, and in both cases FA was larger in males than in females. Sexual selection is supposedly stronger in males, giving rise to sexual dimorphism in horns and body size (males are larger than females), and we can speculate that the difference in FA between sexes is owing to the difference in the strength of sexual selection.

Our results suggest that FA, because of its relationship with blood parameters and, hence, with health, could be used as an estimate of individual quality. This finding might be of interest in conservation biology, because both the management of captive populations and their eventual reintroduction in the wild require the assessment of individual quality. In any case, FA should be used,

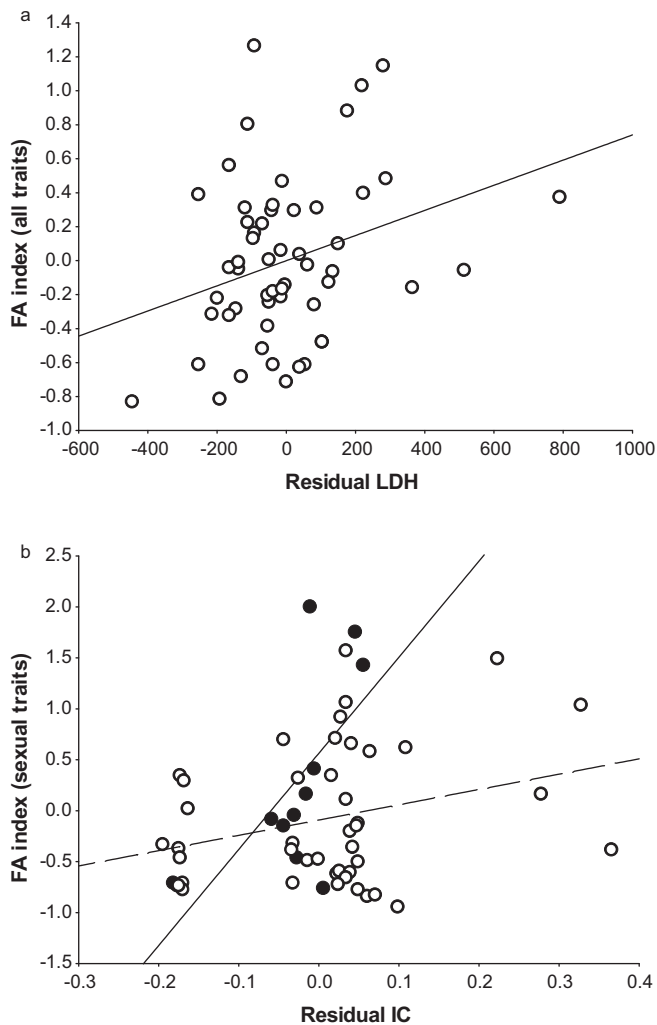


Fig. 3. FA index of (a) all morphological traits in relation to residual LDH (residuals from regressing LDH on inbreeding coefficient (IC)) in *G. dorcas*, and (b) sexual traits in relation to residual IC (residuals from regressing IC on BUN) in males (close circles, solid line) and females (open circles, broken line) of this species. Lines represent the linear fits.

not instead of other estimates of individual quality, but in addition to them.

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