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Plasmodium infections and fluctuating asymmetry among children and teenagers from Senegal

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ABSTRACT

Although fluctuating asymmetry is a sensitive indicator of stress, its links with health remains controversial, especially in humans. Here, we explored for the first time the association between fluctuating asymmetry and malaria infections in humans, from 107 participants involved in a long term medical survey in Senegal. No clear relationship was detected. Depending on traits considered, associations were not significant, or (marginally) significant but not in the same directions. We discuss the possible reasons for the global weakness of the signals detected in this study.

1. Introduction

Fluctuating asymmetry (FA) refers to the non-pathological left–right asymmetry of body traits that are usually left–right symmetrical, i.e. deviations in either direction from perfect bilateral symmetry (Palmer, 1994; Van Valen, 1962; Palmer and Strobeck, 1986). It is classified as a non-directional asymmetry (NDA) because, in spite of individual asymmetries, the average values of left and right sides are not statistically different in the population. At the individual level, for a trait showing FA, both the amount and the direction of bilateral differences are randomly distributed. Antisymmetry is another kind of NDA, where the amount of bilateral difference is more or less constant among individuals. FA is considered as a marker of developmental stability – i.e. the ability to withstand developmental perturbations (and consequently a putative indicator of underlying genetic quality (Møller, 1997). This imprecise expression in the developmental design occurs in most individuals from a large range of species of both plants and animals (see Møller and Swaddle, 1997 for review). Although the nature and amplitude of FA can vary considerably between taxa, traits, and/or types of stress, it has been shown to increase with exposure during ontogeny to genetic perturbations (e.g. inbreeding, deleterious recessives, homozygosity…) and/or to various environmental insults including pollutants, radiation and parasitism (see review by Møller and Swaddle, 1997). FA is thus frequently used as a monitoring tool to reveal environmental stress experienced by organisms (Parsons, 1990, 1992), being sometimes a more sensitive indicator of stress than traditional measures such as mortality, growth rate, fecundity or population density (Leary and Allendorf, 1989; Thornhill and Møller, 1997).

Linking FA and health in humans has been considered as a promising avenue but yielded inconsistent results (see Van Dongen, 2006; Livshits and Kobyliansky, 1991; Van Dongen and Gangestad, 2011 for review), associations being sometimes positive (see Møller, 1999, 2006; Thornhill and Møller, 1997 for review), negative (Gangestad and Thornhill, 1997) or not significant (e.g. Hume and Montgomerie, 2001; Tomkinson and Olds, 2000). For instance, in a recent study Pound et al. (2014) investigated relationships between facial fluctuating FA and detailed individual health histories in a large sample of 4732 individuals

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from a large longitudinal study in South West England and did not support the idea that facial symmetry acts as a reliable cue to physiological health. Conversely, Thornhill and Gangestad (2006) found significant, positive associations between FA and the number of respiratory infections/antibiotic use, suggesting that developmental stability covaries positively with disease resistance. Human health, however, represents a huge and diversified field (e.g. mental and physiological disorders, cardio vascular diseases, ageing, cancers, infectious diseases, etc.), suggesting that despite the considerable amount of study conducted to date, more work is undoubtedly needed to provide a full understanding of the relationships between FA, health and fitness in humans (Penton-Voak et al., 2001; Honekopp et al., 2004; Holtzman et al., 2011; Van Dongen and Gangestad, 2011; Munoz-Reyes et al., 2012).

As far as infectious diseases are concerned, the literature currently suggests that infection in both animals and humans can be a cause of FA, as well as an indicator of susceptibility to pathogens (see Møller, 1999, 2006 for review). Indeed, high levels of developmental instability may make individuals more susceptible to various diseases, including infectious ones, and/or this can also be the outcome of parasite attacks during development, if for instance the activation of the immune system causes increased FA in developing individuals.Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the genus Plasmodium. It is one of the most severe public *health problems* in tropical and sub-tropical areas, being a leading cause of severe disease and death in many developing countries, where young children and pregnant women are the groups most affected (Breman, 2001). In this study, we explored whether infection with *Plasmodium* parasites is associated with increased FA among children and teenagers from Senegal. In tropical Africa, malaria differs from all other regions of the world, having a particular ferocity due to the exceptional vectorial capacity of the three anopheline species (*Anopheles gambiae* s.s., *Anopheles arabiensis* and *Anopheles funestus*) which are endemic in this region of the world (Trape et al., 2014). Although the pattern of illness varies according to the species of *Plasmodium*, patients with malaria usually exhibit high fever and anemia, and can also develop several more or less serious complications (e.g. respiratory distress, pulmonary oedema, encephalopathy, convulsions, splenomegaly, hypoglycemia...) (Bartoloni and Zammarchi, 2012; Mackintosh et al., 2004). Because relapses (especially with *Plasmodium vivax* and *Plasmodium ovale*) and reinfections may occur, frequency of illness at the individual level is elevated in tropical and subtropical African regions, at least until the host develops immunity. For all these reasons, malaria appears as a disease with a high potential to alter developmental stability when it occurs during an organism's development. To explore this avenue, we focused our FA study on young persons because they are both vulnerable to malaria due to an absence of immunity to the parasite, and also because they are predicted to have higher FA levels than adults because rapid growth may make it difficult to maintain symmetry (Mitton, 1993; Wilson and Manning, 1996). This is to our knowledge the first study exploring the links between FA and malaria infection in humans.

2. Materials and methods

2.1. Participants

We conducted our study with 171 participants (77 males and 94 females) aged from 1 to 20 years. All were resident from Dielmo (13°45′N, 16°25′W), Senegal, a village 280 km south east of Dakar and about 15 km north of the Gambian border. Between 1990 and 2012, a prospective longitudinal study of the Dielmo population has been performed to identify all episodes of fever and investigate the host/vector/parasite association of malaria (see Trape et al., 2014 for details). This survey included daily medical surveillance with systematic parasite detection for individuals with fever. This project was initially approved by the Ministry of Health of Senegal and the assembled village population. Approval was then renewed on a yearly basis with written informed consent for individuals enrolled in the project and parents (or adoptive parents, child custodian) for children. Audits were performed regularly by the National Ethics Committee of Senegal and ad-hoc committees of the Ministry of Health, The Pasteur Institutes (Dakar and Paris), and the Institut de Recherche pour le Développement (IRD, formerly ORSTOM).

3. Procedures

3.1. Fever frequency and Plasmodium infections

All households were visited daily from 1990 to 2012, except on Sunday, and nominative information was recorded including the presence of fever or other symptoms. Body temperature was systematically recorded at home three times a week (every other day) in children younger than 5 years of age, and in older children and adults in cases of history of fever or fever-related symptoms (hot body, asthenia, headache, vomiting, diarrhoea, abdominal pain, cough). Blood testing was done for all suspected or confirmed cases of fever, and we provided detailed medical examination, prompt diagnosis and specific treatment for malaria and other diseases, applying the national guidelines of the Extended Programme of Immunization (EPI). With this protocol we could then measure malaria prevalence and density (see below) for each *Plasmodium* species at least quarterly from 1990 to 2012 for all participants. Blood was taken using a finger prick and we examined 200 oil-immersion fields (approximately 0.5 μl of blood). We applied similar procedures when examining thick blood films from patients. We measured the incidence rates of malaria episodes and other causes of fevers as the ratio of the number of fever episodes recorded during a given period divided by the number of followed up person-days under survey during the corresponding period. We counted separately two episodes of fever (including history of fever) if they occurred 15 or more days apart. We attributed fever to malaria when parasite density was higher than an age-dependent threshold calculated for each *Plasmodium* species during the corresponding period according to methods described in Roucher et al. (2012). Maximum threshold values in young children for *P. ovale* and *malariae* clinical attacks ranged from 3800/μl to 2000/μl according to study periods and decreased to 350–300/μl in older adults (Roucher et al., 2014). For *Plasmodium falciparum*, maximum threshold values in young children ranged from 21,500/μl to 10,000/μl and minimum values in older adults from 2000/μl to 500/μl (Roucher et al., 2012, 2014). For infants, thick blood films were taken twice a month up to six months and we attributed fever episodes to malaria when the onset of fever corresponded either to the onset of patent parasitaemia and/or to peaks of high parasitaemia.

3.2. Photographs

Asymmetry was assessed by taking two facial photographs for each individual with a digital camera (Olympus) from a constant distance of 1.5 m at a resolution of 1600 × 1200 pixels. Before being photographed, subjects' hair was removed from the face and glasses were removed. Participants were asked to look straight into the camera and maintain a neutral facial expression with their

mouth closed and eyes opened. This was not always possible given the young age of certain participants; photographs were then not included in the sample (64 photos including 48 females and 16 males) so that the final sample includes 107 participants (61 males and 46 females). All photographs were taken by the same person at the end of the study (C. Roucher). Photos were taken with participants standing. The analysis of facial symmetry from pictures was done on the basis of 12 landmarks that were placed on each side of the face (Fig. 1). Based on these landmarks, we calculated the six following distances: the width of the iris (P1; P2), the width of the eye (endocanthion to exocanthion: P3–P5; P4–P6), the distance between the center of the iris and the base of the nose (center of the iris to subnasale: P1–P7; P2–P7), the distance between the eye corner and the base of the nose (endocanthion to subnasale: P3–P7; P4–P7), the length of the ear (superaurale to subaurale: P8–P10; P9–P11) and the distance between the base of the ear and the middle of the chin (subaurale to gnathion: P10–P12; P11–P12). Each measurement was repeated twice by the same observer. These traits were chosen because they have been commonly used in previous FA studies on humans and have been shown to be largely free of directional asymmetry (DA) and kurtosis (Van Dongen et al., 2008). Landmarks were placed in two independent sessions (i.e. on three separate days) and distances were averaged across sessions to reduce measurement error. All measurements were performed in ImageJ, freely available at http://rsb.info.nih.gov/ij/.

3.3. Statistical analyses

For each trait, the components of asymmetry were estimated in the total population ($n = 107$) following the methodology of Palmer and Strobeck (1986), with the use of CLIC software (Dujardin et al., 2010). The variables showing typical FA (no directional asymmetry) were analyzed separately at the individual level and their sides differences correlated with clinical history. The average of the two measurements was taken as the value of the trait for a given side. Then, the unsigned fluctuating asymmetry of the trait was defined as

$$uFA = 2|(R - L) - (< R > - < L >)|/(R + L)$$

where R and L are the right end left measurements, <R> and <L> the sample averages while |.| denotes the absolute value. The normalizing factor $2/(R + L)$ is the reciprocal of the overall size of the individual.

In order to reduce the dimensionality of the asymmetry data, we performed a principal component analysis (PCA) on the six asymmetry values. The first two components were subsequently used as the response in multiple regressions with the variables of interest (the number of malaria episodes), and with age and sex as control variables. All calculations were performed with the R software (R-core Team, 2013).

4. Results

4.1. Components of asymmetry

Since directional asymmetry is by nature a population feature, and since this study focuses on individual factors, this will not be discussed further. Out of the six characters, two only, width of the eye (endocanthion to exocanthion) and iris did not show any significant directional asymmetry, although they showed a significant non-directional asymmetry (NDA) (see Table 1). The distribution of signed differences indicated this NDA was compatible with fluctuating asymmetry (FA). The remaining variables showed significant directional asymmetry making it difficult to make a clear interpretation about the possible existence of FA.

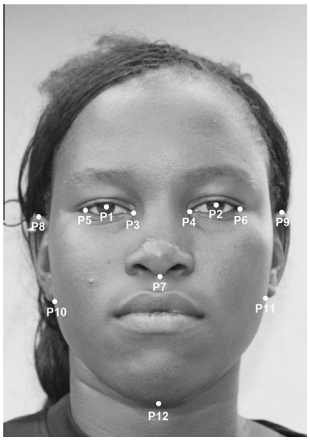

Fig. 1. Landmarks located at both sides of facial photographs. (Photo published with permission). Identification of anthropometric landmarks to determine measurements used: P1/P2, Iris; P3/P4: endocanthion; P5/P6: exocanthion; P7: subnasale; P8/P9: superaurale; P10/P11: subaurale; P12: gnathion.

Table 1
Two-way mixed ANOVA as in Palmer and Strobeck (1986) applied to the total population using variables 1 and 5, with sides (≪side≫) as fixed effects and individuals (≪indiv≫) as random effects, and their interaction (≪side*i≫). For these two variables only, a significant non-directional asymmetry (NDA) was disclosed with no evidence for directional asymmetry (DA). For these two variables, the distribution of signed bilateral difference was in agreement with NDA as being fluctuating asymmetry (FA). Other variables (2, 3, 4, and 6) showed a mixing of DA and NDA (not shown). Note: for these variables 1 and 5, as well as for the remaining ones (2, 3, 4, and 6), no correlation could be detected between the level of between sides differences (R – L) and the estimated size of the individual ((R + L)/2).

Variable 1	Source	SS	df	MS	*F*	Signific.
1	Model	1077721.41	211.00	5107.68	77.73	0.0000
	Indiv	1065285.27	105.00	10145.57	87.48	0.0000
	Aide*i	258.47	1.00	258.47	2.23	0.1385
	Aide*i	12177.67	105.00	115.98	1.76	0.0003
	Residu	13931.38	212.00	65.71		
5	Model	296284.69	211.00	1404.19	260.60	0.0000
	Indiv	295207.35	105.00	2811.50	276.82	0.0000
	Aide*i	10.91	1.00	10.91	1.07	0.3023
	Aide*i	1066.43	105.00	10.16	1.88	0.0001
	Residu	1142.33	212.00	5.39		

4.2. Fluctuating asymmetry and fever episodes

The first two principal components explained 53% of the variance (first component 33%, second component 20%). Fig. 2 shows the projection of the normalized asymmetry values on the first principal plane. Variables age and number of *P. falciparum* fever episodes were also projected, to illustrate their relationship with the principal components and the asymmetry values. Fig. 2 shows clearly that there are two groups of asymmetry variables, the first one, involving asymmetries of eye-to-nose, eye-to-nose-corner and ear-to-chin distances, is close to the first principal component, whereas the second group, involving eye width, iris width and ear height, is close to the second principal component.

Table 2 reports the results of linear regression of the two principal components on the three malaria variables: numbers of episodes of *P. falciparum*, *P. malariae* and *P. ovale* malaria. These three variables were tested separately, and sex and age were included as controlling factors in each model. Similarly, Table 3 shows the results of linear regressions of each trait on the three malaria variables: numbers of episodes of *P. falciparum*, *P. malariae* and *P. ovale* malaria.

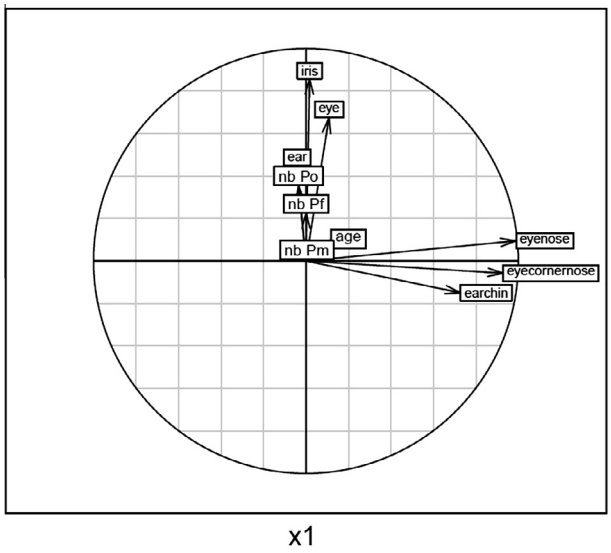

Fig. 2. Projections on the first principal plane of the original symmetry variables (eye, iris and ear sizes, ear to chin, eat to nose and ear to corner of the nose distances). First principal axis is horizontal. Also projected are the variables used in the linear model: numbers of fever episodes of *P. falciparum*, *P. malariae* and *P. ovale* respectively, and age.

5. Discussion

Unlike the conclusions of Van Dongen et al. (2008), most of the characters were affected by directional asymmetry (DA). In our data, accepting the typical association between FA and developmental instability, only the asymmetry of two characters not affected by any significant DA should retain our attention. Interestingly, all the remaining variables showing DA had larger right dimensions, suggesting a possible connection with handedness. However, since they also showed a significant interaction between sides and individuals, their pooled level of individual asymmetry was considered (see Materials and Methods).

Several studies are consistent with the idea that episodes of parasitism during host development cause developmental instability both in animals (Møller, 1992; Polak, 1993; Folstad et al., 1996; Thomas et al., 1998) and humans (Alter, 1966; Kantor, 1994; Wright et al., 1972), and hence enhance FA. However, there are also several studies that fail to detect such a relationship (e.g. Lajeunesse, 2007; Pecinkova et al., 2007) and it is also probable that not all negative results have been published (but see Møller et al., 2005). There are several reasons for why FA is not necessarily correlated to parasitism, ranging from the fact that parasites may not be responsible for developmental instability and/or because many factors might blur the relationship between FA and developmental instability (see Van Dongen, 2006 for review).

Table 2
Regression of the first principal components (a) and second principal components (b) vs. number of *Plasmodium* sp. fever episodes.

Covariate	Sign of slope	*P*-value
(a)		
P. falciparum	+	0.33
P. malariae	+	0.53
P. ovale	+	0.28
(b)		
P. falciparum	–	0.033
P. malariae	+	0.61
P. ovale	–	0.00022

In this study, despite an impressive and continuous medical survey, no clear relationship was detected on the relationships between FA and fever episodes due to *Plasmodium* infections, since, depending on traits considered, associations were not significant, or (marginally) significant but not in the same direction. Indeed, two measurements of FA (eye and iris) are positively correlated with *Plasmodium* fevers, while for eyecornernose a negative correlation is observed. Positive correlations are in accordance with the prediction that FA should increase with infections, being either a cause or a consequence (see Introduction). It is however unclear why one measurement concerns fevers due to *P. falciparum* and the other due to *P. ovale*. The negative correlation is more problematic to explain. One possibility is that FA on eyecornernose is correlated to significant health disorders and as a consequence only individuals with low FA levels on this trait are able to survive to repeated episodes of *Plasmodium* infection. However, to avoid speculative interpretations on this finding, a more complete medical survey would be needed.

Several reasons can be invoked to explain the global weakness of the signals detected in this study. First it is possible that the power of the statistical analysis is low. If we accept a power of 80% and a significance level of 5%, then if the true effect is small with $r = 0.10$, as is typically the case for asymmetry studies, a sample size of 700 would be required to accept the null hypothesis. This raises the difficulty of accepting the null hypothesis. Second, the variables used in the study were the distances between anatomical landmarks, ignoring their relative positions. An approach based on landmarks coordinates, i.e. combining both kinds of information (Rohlf and Marcus, 1993), could be more powerful to detect asymmetries. In addition, it might be that *Plasmodium* infections, despite their well know detrimental effects on health, are not associated to developmental instability. It is also possible that their effects on developmental stability are small compared to other stress incurred by people living in this area. Unless we control for these stronger confounding effects, it is difficult to highlight the influence of *Plasmodium* fevers on FA. Finally, even though *Plasmodium* infections would be associated to developmental instability, it is possible that the number of malaria-induced fevers remains a poor estimate of the detrimental effect of *Plasmodium* on developmental stability. For instance, a significant proportion of children in Dielmo harbor *Plasmodium*

Table 3
Regression of unsigned fluctuating asymmetry vs. number of *P. falciparum* (a), *P. malariae* (b) and *P. ovale* (c) fever episodes. Adjusted for age.

Trait	Sign of slope	*P*-value
(a)		
Eye	+	0.038
Eyenose	–	0.43
Eyecornernose	–	0.040
Ear	+	0.057
Earchin	+	0.64
Pupil	+	0.73
(b)		
Eye	+	0.84
Eyenose	–	0.77
Eyecornernose	+	0.87
Ear	–	0.73
Earchin	–	0.61
Pupil	–	0.57
(c)		
Eye	+	0.38
Eyenose	–	0.44
Eyecornernose	–	0.12
Ear	+	0.23
Earchin	+	0.88
Pupil	+	0.014

without presenting signs of clinical malaria and are considered asymptomatic cases. Until the link between asymptomatic infections and health components are better understood, we cannot exclude that a positive association between *Plasmodium* infection and FA exists but that it is hidden when only fever episodes are considered. The fact that significant associations concern *P. falciparum* and *P. ovale*, but not *P. malariae* remains unclear but could be due the fact that *P. malariae* infections in Dielmo were low grade, long lasting, almost always associated with *P. falciparum* infections and rarely cause of fever (Trape et al., 1994).

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