Editorial Manager(tm) for Naturwissenschaften
Manuscript Draft

Manuscript Number: NAWI-D-11-00017R2

Title: Geometric morphometrics as a tool for improving the comparative study of behavioural postures

Article Type: Original Article

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Abstract: Describing postures has always been a central concern when studying behaviour. However, attempts to compare postures objectively at phylogenetical, populational, inter- or intra-individual levels generally either rely upon a few key elements or remain highly subjective. Here we propose a novel approach, based on well-established geometric morphometrics, to describe and to analyse postures globally (i.e. considering the animal's body posture in its entirety rather than focusing only on a few salient elements, such as head or tail position). Geometric morphometrics is concerned with describing and comparing variation and changes in the form (size and shape) of organisms using the coordinates of a series of homologous landmarks (i.e. positioned in relation to skeletal or muscular cues that are the same for different species for every variety of form and function and that have derived from a common ancestor, i.e. they have a common evolutionary ancestry, e.g. neck, wings, flipper /hand). We applied this approach to horses, using global postures 1) to characterise behaviours that correspond to different arousal levels, 2) to test potential impact of environmental changes on postures. Our application of geometric morphometrics to horse postures showed that this method can be used to characterise behavioural categories, to evaluate the impact of environmental factors (here human actions) and to compare individuals and groups. Beyond its application to horses, this promising approach could be applied to all questions involving the analysis of postures (evolution of displays, expression of emotions, stress and welfare, behavioural repertoires...) and could lead to a whole new line of research.

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Yours sincerely,

Carole Fureix

Geometric morphometrics as a tool for improving the comparative study of behavioural postures

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Describing postures has always been a central concern when studying behaviour. However, attempts to compare postures objectively at phylogenetical, populational, inter- or intra-individual levels generally either rely upon a few key elements or remain highly subjective. Here we propose a novel approach, based on well-established geometric morphometrics, to describe and to analyse postures globally (*i.e.* considering the animal's body posture in its entirety rather than focusing only on a few salient elements, such as head or tail position). Geometric morphometrics is concerned with describing and comparing variation and changes in the form (size and shape) of organisms using the coordinates of a series of homologous landmarks (*i.e.* positioned in relation to skeletal or muscular cues that are the same for different species for every variety of form and function and that have derived from a common ancestor, *i.e.* they have a common evolutionary ancestry, *e.g.* neck, wings, flipper /hand). We applied this approach to horses, using global postures 1) to characterise behaviours that correspond to different arousal levels, 2) to test potential impact of environmental changes on postures. Our application of geometric morphometrics to horse postures showed that this method can be used to characterise behavioural categories, to evaluate the impact of environmental factors (here human actions) and to compare individuals and groups. Beyond its application to horses, this promising approach could be applied to all questions involving the analysis of postures (evolution of displays, expression of emotions, stress and welfare, behavioural repertoires...) and could lead to a whole new line of research.

Keywords: posture analysis; geometric morphometrics; innovative methodological application ; horses; ethology

INTRODUCTION

From Darwin (1872) to Platon (Frere 1998), descriptions of animal behaviour have always been based on postures. Because body expression is one way for animals to convey emotions, Darwin based his concept of continuity of behaviour between species on the continuum of postural expressions of emotions. All through the history of animal behaviour research, description of postures has been central (Guyomarc'h et al. 1987) and fundamental for defining behavioural repertoires (Baerends 1972), evaluating individual or population differences or the evolution of behaviour (Wickler 1967). More recently, postures have again been considered as a major tool for evaluating stress and emotional states (*e.g.* Beerda et al. 1999; Reefmann et al. 2009) or detecting anxiety (Lepicard et al. 2003).

However, attempts to compare postures objectively at these different levels (definition of behavioural repertoires, evolution of behaviour, impact of stress…) generally rely only upon a few key elements, only salient parts of the body like the head and tail, and/or remain highly subjective (*i.e.* mere visual evaluation with no quantifiable details). For instance, descriptions of blue tits' (*Cyanistes caeruleus*) attack / flight postures (Stokes 1962) were based on 36 correlations between 9 elements such as wings, beak, and tail, whose positions were evaluated arbitrarily. Baerends & van der Cingel (1962) compared the "snap display" of common herons, *Ardea cinerea,* to displays of other species on the basis of measurements of neck angle, head orientation, and tibio-tarsal angle, but such measurements were also focused on a few salient elements and did not consider the animal's body posture in its entirety (*i.e.* did not provide a global posture assessment).

Current researchers are faced with the same difficulties when comparing postures. Descriptions of individual profiles of male quail (*Coturnix japonica*) displays have used a combination of elements (*e.g.* legs bent/ extended, head stretched and body or wings lowered, Lumineau et al. 2005), which do not provide a global overall posture assessment. Estimation of welfare and of anxiety are also based on a few salient elements, such as tail angle and trunk height in anxious mice (Lepicard et al. 2003). When used, global posture assessments are based on very coarse postural elements, *e.g.* the animal is merely recorded as lying or standing (Huzzey et al. 2005; Krawczel et al. 2008; Xin 1999), or remain subjective (*e.g.* the low posture in stressed dogs, where "the position of the tail is lowered (…) and the legs are bent" compared to "the breed specific posture shown by dogs under neutral conditions", Beerda et al. 1999).

Ethologists try to cope with the limitations of subjective categorisation by using inter-subjective agreement, asking different observers to categorise the same items. Yet these procedures do not guarantee the reproducibility of the measures, for instance between laboratories or groups of animals. Moreover, despite the importance of "postural behaviour as an integrated biological sensor" (Xin 1999), no satisfying global representation, leading to appropriate statistical comparisons of body postures in their entirety, has yet emerged. Here we argue that systematic quantitative analyses, using clear anatomical landmarks, would both 1) allow one to study posture as a whole and 2) improve the objectivity and reproducibility of postural measures by quantifying the amplitude of their variation rather than recording their mere occurrence. Automated behaviour and movement detection has been used in animals, as in kinematic studies using markers stuck or painted on animals, or surgically implanted (*e.g.* in horses: Licka and Peham 1998, Faber et al. 2000, Haussler and Erb 2006, Peham and Schobesberger 2006, Hobbs et al. 2010; in ferrets: Kafkafi and Golani 1998). However, one of the major limitations of this methodology is that animals are tested in highly artificial situations, moving in front of fixed cameras in a calibrated environment since fixed "positions" defined beforehand in the environment are needed to obtain coordinates to use as a reference frame for measurements in the tracking program. Here we propose a novel approach to describe and to analyse global body postures on the basis of geometric morphometrics (hereafter GM) recognised in biology for its descriptive power and its high statistical power (Adams et al. 2004). We applied GM for the first time to the study of posture in domestic horses *Equus caballus*, testing whether GM could be used for detecting different body posture. The horse is a highly appropriate model to study the application of GM tools to ethological questions for several reasons. First, visible postures associated with different activities or behaviour have been described previously by Kiley-Worthington (1976), but only head and tail positions were considered. Here we predicted that GM would allow us to describe and to analyse global body posture variation as a function of behaviour. Second, horses have recently been shown to be sensitive to subtle cues humans display while interacting with animals, for instance in relation to their attentional (Proops & McComb 2010; Takimoto & Fujita 2008) and emotional states (Keeling et al. 2009; Hama et al. 1996). Such a high sensitivity to humans allows one to predict that horses' posture could vary according to the presence of people (*i.e.* while a horse is interacting with humans, *e.g.* being led) or their absence (*i.e.* while a horse is performing spontaneous behaviour, *e.g.* locomotion in a pasture). Finally, working conditions such as being ridden may lead to undesirable postures at work (neck height and curve) leading to the same long-term negative effects, such as the occurrence of chronic back problems (Lesimple et al. 2010). This may influence posture, as horses with back problems have been reported to present a flat and rigid whole back (Cauvin 1997; Faber et al. 2000). Thus, horses provide an interesting model to test the impact of living conditions (*e.g.* working conditions) on posture by comparing domestic horses kept under natural conditions (optimal for their welfare) and horses from riding schools, kept under conditions known to have some negative impact on their welfare (*e.g.* poor working conditions leading to potential vertebral problems, social isolation in boxes, time-restricted feeding practices..., Mc Greevy et al. 1995; Cooper et al. 2000; Lesimple et al. 2010). We thus predicted that horses' global posture would vary according to living conditions.

INTEREST AND CONDITIONS OF APPLICATIONS OF GEOMETRIC MORPHOMETRICS

Usually GM analyses morphological differences and changes in organisms (*e.g.* in proportions and respective positions of skeletal features) using the coordinates of a series of homologous reference points ("landmarks") that can then be used to compare specimens. In addition to statistical analysis of morphological changes, GM also provides a way to draw pictures of morphological transformations, *i.e.* to *visualize* one morphology transforming into another by gradually moving a cursor from one morphology to another in the software. Through analyses of shape disparity (*i.e.* variety within a group of species as the outcome of evolutionary processes) and variation within a single population, GM can tackle different types of questions related to the skeleton such as taxonomic affiliation (*i.e.* whether populations are drown from multiple species, and, if so, by what morphological variable(s) they are most effectively discriminated), phylogenetic relationships among taxa or evolutionary issues. However, GM has been used only in a limited number of posture-related studies: morphological variations in the seahorse vertebral system (Bruner & Bartolino 2008); geometrical analysis of footprints in addition to baropodometrical analysis (Bruner et al. 2009). To our knowledge, no study using GM has been directly performed on the postures of living animals.

Zelditch et al. (2004) explained the rationale and applications of these methods in detail, so we only summarise below some basic principles. The theory of shape underlying GM enables a clear distinction between the notions of shape and of scale (size): the calculation of "centroid size" (the sum of distances between every landmark and their centroid) provides a measure of shape independent of size. Two objects of different size are considered similar in shape when they appear identical after filtering out effects of location in space, rotation and scale (*e.g.* with generalised least squares Procrustes superimposition), meaning in our case that shapes of living individuals differing in size (*e.g.* breed-related differences) can be compared easily.

It is of interest that the geometry of a biological structure can thus be analysed as a whole using landmark coordinates. Patterns emerge from the analyses, allowing analysis of the overall posture rather than focusing independently only on salient elements (*e.g.* identifying variations that could occur in different parts of an individual, such as the back and the croup, rather than focusing on the angle between head and body). The analysis itself helps to reveal which variables (distances, relative positions or proportions) are meaningful for the biological questions addressed, potentially highlighting unsuspected subtle postural variations.

Criteria for choosing landmarks for GM are: (1) homologous anatomical loci, (2) consistent relative topological positions, (3) adequate morphological coverage to take into account shape variations, for instance at least three landmarks are required to study a bend, (4) reliable repeatability and (5) all landmarks in the same two dimensional plane (Zelditch et al. 2004). A problem related to these criteria is that complete sets of homologous landmarks are usually required for comparison, meaning that only sufficiently morphologically similar organisms (allowing similar sets of landmarks) can be used in interspecific comparisons. Emerging GM approaches now enable comparisons including 2D and 3D non-homologous landmarks (Bookstein 1997; Gunz et al. 2005).For instance, a specific posture associated with a given environment (*e.g.* head and back position when standing under non friendly-to-welfare breeding conditions) could be investigated across large mammals (*e.g.* horses, cows, …) but not on highly morphologically different organisms such as mammals and poultry.

Finally, an important general concern is the choice of the reference points: what are *relevant* GM landmarks? Blind use of any accessible landmark risks introducing noise and possibly biasing elements in the analysis, via traits correlated with uncontrolled parameters. Conversely, and except for questions already analysed in depth and for which relevant parameters have been accurately documented, restrictive use of a few landmarks focusing on some part of an animal's body could overlook relevant morphological information. Exploratory approaches must use an extended series of landmarks, which can be restricted (or not) later after analysing each landmark's contribution to the shape.

THE STUDY ON HORSES: MATERIAL AND METHODS

Subjects and behaviour

Experiments complied with current French laws (Centre National de la Recherche Scientifique) related to animal experimentation and were in accordance with the European directive 86/609/CEE. No licence / permit / institutional ethical approval were needed. Animal husbandry and care were under the management of a private owner (study 1) or the riding school staff (study 2). This experiment involved only horses in the "field" (no laboratory animals).

We studied two samples of horses kept under different conditions (natural conditions in study 1, horses from riding schools in study 2), allowing us to characterise postures in relation to categories of spontaneous behaviour and to evaluate the impact of environmental factors (human actions, general living conditions: housing, feeding and working conditions).

Study 1

This study aimed, by applying GM tools to horses' postures, to characterise postures in relation to categories of spontaneous behaviour and to evaluate the impact of environmental factors, here human actions (*i.e.* being led: walking and standing). This study included 6 domestic horses kept under natural conditions for more than 10 years, stable social groups year-round in 1-2ha natural pastures, fed grass and hay *ad libitum* during winter (no industrial pellets) and not regularly exercised (2 geldings, 4 stallions; 13-20 years old; French

Saddlebred cross, Haflinger and mixed breeds). Horses were observed performing various spontaneous activities known to be related to different arousal levels (locomotion: slow exploration and sustained walk; motionless behaviour: rest and observation, Table 1) and while interacting with an experimenter (being led: walking and standing motionless near an experimenter; the same 2.6 m long and 600g lead rope was used in all interactions). The experimenter, with whom the horse was not familiar beforehand, did not talk to the horse, stayed on the horse's left side and held the lead rope slackly at a predefined distance from the horse's head (1m), so that the experimenter never pulled the rope nor the horse's head.

Study 2

This study aimed to give a first evaluation of the impact of general living conditions (housing/feeding/working conditions) on horses' postures, again by applying GM tools. In order to address this issue, 63 horses from three riding schools were observed in addition to the study 1 horses in order to compare their postures. The horses from riding schools comprised 46 geldings and 17 mares, 5-20 years old, kept singly in 3 m * 3 m individual straw-bedded boxes, fed industrial pellets (mainly composed of wheat bran: 30%, barley: 28%, flour of alfalfa: 10%, palm kernel: 10%, soya bean: 10%, oats: 6%; treacle, corn, calcium carbonate, sodium chloride, vitamin A, D, E; copper sulphate) 3 times a day and hay once a day, exercised in riding lessons for 4-12 hours per week with at least one free day. Sixty-seven percent of the horses were French Saddlebreds, equally distributed among schools. The other horses belonged to various breeds or were unregistered animals. These riding school horses were included in a larger project evaluating horses' welfare using a multidimensional approach, involving health-related (*e.g.* vertebral state assessment), physiological, behavioural but also postural measures of the animal's welfare state. As previously described, data concerning horses being led (walk and stand, same lead rope as in study 1) were recorded. Spontaneous activities (walks, rest...) could not be evaluated here because the horses were confined in boxes, thus preventing the experimenter from taking pictures perpendicularly and far away enough from the horse (cf. data recording).

Data recording

Eight landmarks (self-adhesive red felt discs, 34 mm in diameter, visible on all coat colours) were stuck onto the horse's right side. The landmarks were placed in a sagittal plane in relation to skeletal or muscular cues (thus enabling consistent reproduction of positioning) from head to croup along the spine (Fig. 1). Landmarks were placed on: the nasal bone under the eye, 2 cm in front of the zygomatic process (landmark 1); the temporo-maxillary joint (landmark 2); the atlas (landmark 3); the trapezium cervical ligament (landmark 4); the cervico-thoracic (landmark 5); the thoraco-lumbar (landmark 6) and the lumbo-sacral (landmark 7) junctions; and the first coccygeal vertebra (landmark 8) (Fig. 1).

Following Huard (2007) arguing that "only the animal's figurative body should be analysed due to limb mobility" when he applied GM tools to representations of Equidae in cave paintings, we voluntarily excluded limb positions recording. Indeed, limb movements inherent to locomotion could introduce too much noise into the analysis. Horses' postures were recorded using photographs taken perpendicularly 10m ± 1m from the horse (digital camera Canon EOS 20D, zoom lens 50mm to limit perspective distortions). All data were recorded by the same experimenters (E.S. – taking pictures and C.F. – leading the horses; see below). Data recording took place between 08.00 a.m. and 06.00 p.m. during a 3-week period for private owners' horses or a 2-day period at each riding school (in all schools during quiet periods, with no riding lessons). Horses kept under natural conditions were photographed 20 times in slow exploration walk, 20 times in sustained walk, 20 times while walking being led by an experimenter, 10 times while resting, 10 times while observing and 10 times while standing held motionless near an experimenter. Thus each animal was photographed 20*3 times in locomotion and 10*3 times while performing motionless behaviours (yielding 90 photographs for each horse). In this preliminary approach, we took more photographs of locomotive than of static behaviours, as we assumed that intra-individual postural variations would be greater for locomotor behaviours. At the riding schools, horses were photographed on average 4.5 (± 0.9) times while walking led by an experimenter and 2.4 (± 0.8) times while standing motionless near an experimenter. Riding school horses were not always available for observation, yielding different numbers of photographs per horse in this first approach (walking: from 2 to 7, and standing: from 2 to 5 per horse).

Data and statistical analyses

In all cases, data of landmark coordinates were extracted from photographs using Tps software (TpsDig2, TpsUtil) and analysed by Generalized Procrustes Analyses using R2.9.2 and TpsRelW free software (R libraries: scatterplot3d, shapes and ade4, F3class command for the graphics). Briefly, landmarks were digitised by only one experimenter (ES, previously trained to this specific set of landmarks) from the photographs using tpsDig software, and then files were loaded from the tpsDig program into another tps software (tpsUtil) to define the sliders (*i.e.* the links between landmarks, creating the shape) and to save the sliders file. Then both files from the tpsDig program and the sliders file were loaded into the tpsRelw and R2.9.2 software to start shape analysis. Thus, Generalized Procrustes Analyses (allowing comparisons of shapes after filtering out effects of location in space, rotation and scale, for more statistical details see Zelditch et al. 2004) and Principal Component Analysis were conducted to identify postures in relation to behaviour (study 1) or to groups of horses according to their general living conditions (study 2). Data on aligned specimens filtering out effects of location in space, rotation and scale were also extracted from tpsRelW and R2.9.2

to conduct a Multivariate Analysis of Variance (MANOVA). MANOVA allows statistical discrimination of postures in relation to behaviour or to groups of horses, taking into account all the landmark coordinates (*i.e.* the global shape of the horse). MANOVAs were conducted using Statistica© 7.1 software (accepted *P* level at 0.05). Some slight postural variations appeared for a given horse performing a given behaviour, probably due to the transitional characteristic inherent to behavioural responses (compared with bones). However, it could be overcome by taking several pictures of a horse performing the behaviour and do not prevent for statistically identifying inter-individual and inter-group variations (see results). Gender differences in postures investigated in study 2 revealed no significant difference between mares and geldings (MANOVA, $F_1 = 1.86$, $p > 0.05$). The difference stallions / geldings (study 1) could not be statistically investigated here due to low number of geldings ($n = 2$), but no sexual shape dimorphism was apparent between these two groups.

THE STUDY ON HORSES: RESULTS

Postures in Relation to Behaviour

The generalised Procrustes analyses allowed us to characterise postures associated with given behaviours at the individual and group levels. Multivariate analysis (PCA) identified specific postures in relation to behaviour for each individual horse (MANOVA for each horse: $F_{10, 168} = 19.44$ to 38.94, $p < 0.001$ in all cases). These inter-behavioural postural variations mainly concerned horses' neck height: horses' necks were highest when they were standing observing and lowest for exploratory walking (Fig. 2a and b). Low arousal behaviour postures (standing observing, standing near a human, resting) showed some similarities but were distinct (on the left side of the graph Fig. 2a) and they clearly differed from active behaviour postures (walking spontaneously, being led – on the right side of the graph Fig2a). Exploratory walk posture, characterised by lower neck and wide head-neck angle, clearly differed from all the other postures. It is of interest that the horse's posture when being led by an experimenter differed from spontaneous locomotion (*e.g.* with a higher neck while walking spontaneously, Fig. 2a and b). Moreover, jaw-neck angles appeared narrower in spontaneous behaviour postures than when led by an experimenter.

Individual postural differences emerged for given behaviours (Fig.3), showing that this method could be used for analysing subtle individual postural variations. Nevertheless, each behavioural category could still be discriminated at the group level as inter-individual variability was lower than inter-behavioural variability (MANOVA, $F_{60, 2452} = 18.48$, $P < 0.001$).

As the neck is the horses' most mobile area, a generalised Procrustes analysis was also conducted independently on back landmarks only (landmarks 5 to 8) to test for a so-called "Pinocchio effect" whereby one prominent feature can invalidate generalised Procrustes approaches and give misleading false positives. However, postures (without neck data, leading to less "biological" meaning about the horse posture, but necessary to control for a Pinocchio effect due to the prominent horses' neck mobility), could still be associated with different behaviours, both at the individual and the group levels (MANOVA, $F_{60, 2452} = 25.10$, $P < 0.001$).

Impact of Environmental Factors on Postures

Postures of horses kept under natural conditions clearly differed from postures of riding-school horses (MANOVA, standing motionless: $F_{36, 570} = 22.51$, walking: $F_{36, 1150} = 45.40$, $P < 0.001$ in both cases) even though the same behaviours were considered. Inter-group postural variations mainly occurred in terms of horses' neck and back roundness (Fig. 4a and b). Thus, horses kept under natural conditions held their necks higher and their backs were rounder than those of horses from riding schools. It is of interest that excluding horses kept under natural conditions, postures could still be discriminated between riding schools (MANOVA, standing motionless: $F_{24, 268} = 7.45$, walking: $F_{24, 540} = 11.69$, $P < 0.001$ in both cases): horses from riding school B had on average straighter and flatter postures than horses from the other riding schools (Fig. 4a and b).

DISCUSSION

Application of GM analysis to horse postures showed that this method can be used to characterise behavioural categories for intra- and inter-individual comparisons, to evaluate the impact of environmental factors and to compare individuals, groups or populations.

At the group level, postural variation occurred in respect of neck and back roundness that were higher in horses kept under natural conditions than in horses from riding schools, both for static and locomotor behaviours. Several factors could partly explain this inter-group variation, such as breed, sex (although no sexual shape dimorphism was apparent in this study) and living conditions (living in box / in pasture, socially isolated / in a group, regularly exercised / not regularly exercised). We propose that exercise (*i.e.* riding) is likely to be a variable of major interest. Repeated exercise is known to impact the physical state of the horse, modifying its kinematics and muscular development (*e.g.* Ödberg & Bouissou 1999; Biau & Barrey 2004; von Borstel et al. 2009), which is likely to influence the horses' posture. In addition, incorrect riding techniques may be a potential source of back problems in horses (*e.g.* Cauvin 1997, Lesimple et al. 2010). Manual examination of vertebral states, based on bony and soft tissue manual palpation of localised regions of vertebral stiffness based on spinal mobilisation and palpable areas of muscle hypertonicity (details in Lesimple et al. 2010; Fureix et al. 2010) had been carried out on the same sample of horses. This previous examination showed that most riding school horses (73%) were severely affected by vertebral problems, while only 27% of the horses could be considered either totally unaffected (15%) or slightly affected (*i.e.* one slightly affected vertebra, 12%) (Fureix et al. 2010). Conversely, horses kept under natural conditions were exempt from such problems (Fureix et al. unpublished data). This suggests that vertebral problems may also be involved in inter-group postural variations. It has been reported that horses with back problems present gait anomalies when their whole back appears flat and rigid (Cauvin 1997; Faber et al. 2000). This hypothesis could also be supported by the fact that postures could still be discriminated between horses from different riding schools (with similar sex ratios and breeds). Indeed, horses from riding schools appear to differ in relation to the occurrence of vertebral problems (Lesimple et al. 2010) and health-related parameters (Fureix and Hausberger unpublished data). Accordingly, the horses from the riding school with the highest rates of vertebral and health-related problems also presented the straightest and flattest postures. In this exploratory study, statistical correlations between vertebral problems and postural data were not tested further due to the number of other variables (housing, feeding conditions, sex…etc.) which could explain inter-group postural variation in addition to the inter-group differences in vertebral states. However, our results here raise new questions about the potential impact of vertebral problems on postures, which are currently under investigation in a large-scale study (Seneque et al. in prep).

Beyond their application to horses, these promising results show that this method can be used to discriminate groups of animals, even though methodological improvements need be added before drawing conclusions concerning the impact of environmental factors (horses kept under natural conditions and coming from riding schools were not equally represented in this exploratory study) and individual characteristics (such as age, sex and breed) on postures. On-going studies are currently using this method on more balanced samples to address questions concerning welfare state and vertebral problem impact (Seneque et al. in prep), emotional level and impact of human positioning on horses' behaviour (Fureix et al. in prep).

Note that the experimenter was not allowed to pull on the rope when leading the horses, so that she was not likely to influence posture by a direct action on the horse's head. Nevertheless, one could address the question of the weight of the lead rope *per se*, which could partly explain the lower neck when horses are led compared to spontaneous walk, for instance by fitting free-ranging horses with the same equipment. However even if the relative impacts of human / lead rope presence *per se* on horses' postures remains to be investigated, GM appears to be effective for detecting different body postures as a function of different contexts.

Markers stuck on animals have been used in kinematic studies (*e.g.* horses: Licka and Peham 1998, Faber et al. 2000, Haussler and Erb 2006, Peham and Schobesberger 2006, Hobbs et al. 2010; ferrets: Kafkafi and Golani 1998). However, a major limitation of this method is that animals must move in front of fixed cameras in a calibrated environment (*i.e.* need for fixed "positions" defined beforehand in the environment to obtain coordinates to use as a reference frame for measurements in the tracking program). Consequently, it is difficult to study free-ranging or slightly constrained subjects, as the animal may move out of the reference frame. This could be overcome in a small arena equipped with several cameras, but prevents observation in a large space, such as the usual pasture for horses or other stock. Insomuch as animals can be fitted before hand with landmarks (as in kinematic investigations), the protocol used here, *i.e.* taking pictures orthogonally sideways, appears to be easier to implement in the field, as it does not require a calibrated space, provided that there are no visual obstacles between the photographer and the subject. Procrustes adjustment corrects for moderate variations in camera/horse distance, as it allows comparisons of shapes after filtering out effects of location in space, rotation and scale (Zelditch et al. 2004). Thus the experimenter could take pictures from various distances (*e.g.* here 10m ± 1m), so as not to interfere with the animal's spontaneous behaviour (as long as the pictures were taken orthogonally sideways, which is facilitated by the possibility for the experimenter to move freely from a calibrated environment). Thus this protocol allows free-roaming or slightly constrained subjects complete freedom of movement, a major advantage in studying posture in relation to spontaneous behaviours (outside the context of a human / animal interaction).

Beyond its application to horses, this approach adds an innovative way to standardise methods related to measuring and interpreting postures in relation to behaviour. This promising use of GM for ethology, and behavioural research in general, could open a broad field of investigation, adding a complementary tool, in fundamental ethology, physiology, behavioural ecology and evolutionary biology, involving inter-individual, inter-population or inter-specific comparisons in relation to context or internal state.

ACKNOWLEDGEMENTS

The authors are grateful to the managers of the riding schools for allowing them to work with their horses, and all the staff for their help and understanding. We are grateful to Dr Ann Cloarec, Dr Carol Sankey and Prof Adrian Craig for correcting the English of this manuscript. This work was supported by the Caisse Centrale de la Mutualité Sociale Agricole. This manuscript benefitted from comments from three anonymous reviewers.

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FIGURES CAPTIONS

Fig 1 The eight landmarks. Landmarks were stuck onto the horse's right side and placed in relation to skeletal or muscular cues on: the nasal bone under the eye, 2 cm in front of the zygomatic process (landmark 1); the temporo-maxillary joint (2); the atlas (3); the trapezium cervical ligament (4); the cervico-thoracic (5); the thoraco-lumbar (6) and the lumbo-sacral (7) junctions and the first coccygeal vertebra (8). Photographs were taken perpendicularly from the horse performing various behaviours, and data of landmark coordinates were extracted from photographs using GM software and analysed by Generalized Procrustes Analyses, allowing to describe and to analyse global body posture variation (*e.g.* as a function of behaviour).

Fig 2 Postures in relation to behaviours at the individual level (here horse E2)

a) Principal component analysis (Thin plate spline, TPS, relative warp analysis) based on TPS shape parameters. Barycentres of the observed postures (letters, see b for the representation of corresponding postures) and distribution values (showing the range of variation between observed postures for a given behaviour, represented on the graph by a circle around letters) are represented for each behaviour: **E** = exploratory walk, **O** = observation, **R** = rest, **S** = standing motionless near an experimenter, **W** = sustained walk, **We** = walk led by the experimenter. **b)** Corresponding postures as depicted by TPS deformation grids, showing the mean horse's posture for a given behaviour. For instance, the first grid (at the top) represents the horse's mean posture while standing observing (localised by the letter O on the left of the Principal Component Analysis representation). Axis 1 explained 78.60% of postural variation. Inter-behavioural postural variation mainly occurred in terms of horses' neck height that varied from the highest when a horse was standing observing (O, grid at the top of the list) to the lowest in exploratory walking (E, lowest grid).

Fig 3. Postures in relation to individual horses (here for the behaviour sustained walk)

Principal component analysis (Thin plate spline, TPS, relative warp analysis) based on TPS shape parameters. Barycentres of the observed postures (E1, E2, E3...) and distribution values (showing the range of variation between observed postures for a given behaviour, represented on the graph by a circle around letters) are represented for each horse. Corresponding postures as depicted by TPS deformation grids, showing the mean individual horse's posture when performing sustained walk. Axis 1 explained 52% of postural variation. Inter-individual postural variation mainly occurred in terms of horses' neck and croup height in relation to the back position, which varied from the highest for the horse E2 (left side) to the lowest for the horse H1 (right side). Thus individual postural differences emerged for given behaviours, showing that this method could be used for analysing subtle individual postural variations. However, note that each behavioural category could still be statistically discriminated as inter-individual variability was lower than inter-behavioural variability.

Fig 4 Postures in relation to behaviours at the group level.

Principal component analysis (Thin plate spline, TPS, relative warp analysis, axes 2 and 3) based on TPS shape parameters and corresponding postures (black lines) as depicted by deformation grids. Barycentres of the observed postures (letters) and distribution values (showing the range of variation between observed postures for the behaviour, represented on the graph by a circle around letters) are represented for **A**: horses kept under natural conditions, **B**, **C and D**: horses from riding school B, riding school C and riding school D. Mean postures (representation extracted from TPS deformation grids) are represented for each population of horses (from A to D) while **a)** standing motionless near the experimenter and **b)** walking led by the experimenter.

Axis 1 (not shown) explained respectively 50.80 % of the postural variation when horses stood motionless and 49.70% when horses walked (variation occurred in neck height). For both behaviours considered, the inter-group postural variation occurred mainly in horses' neck height and back roundness: horses kept under natural conditions (A) had higher necks and back roundness than horses from riding schools (B, C and D). Distribution along axis 3 revealed that horses' postures also differed among riding schools: horses from riding school B had on average straighter and flatter posture than those from riding schools C and D.

TABLE

Table 1. Description of spontaneous activities known to be related to different arousal levels (used here for assessing horses' postures according to behaviour, applying tools from geometric morphometrics). Please note that these behaviours were only recorded for horses kept under natural conditions (in stable social groups in pasture). Adapted from McDonnell (2003)

Name	Description
Slow exploratory walk	The horse walks slowly with its neck horizontal or below the horizontal, ready to stop and sniff the ground or the wall. This is the characteristic slow walk of a quiet horse in a calm situation. There is no muscular tension.
Sustained walk	The horse walks energetically and looks forward or around.
Standing resting	When resting, the horse stands with its eyes at least partly closed. Its muscles relax and its lips can get droopy. The horse can be standing on only three legs.
Standing observing	The horse stands still, with head and ears oriented towards the object.

Figure1

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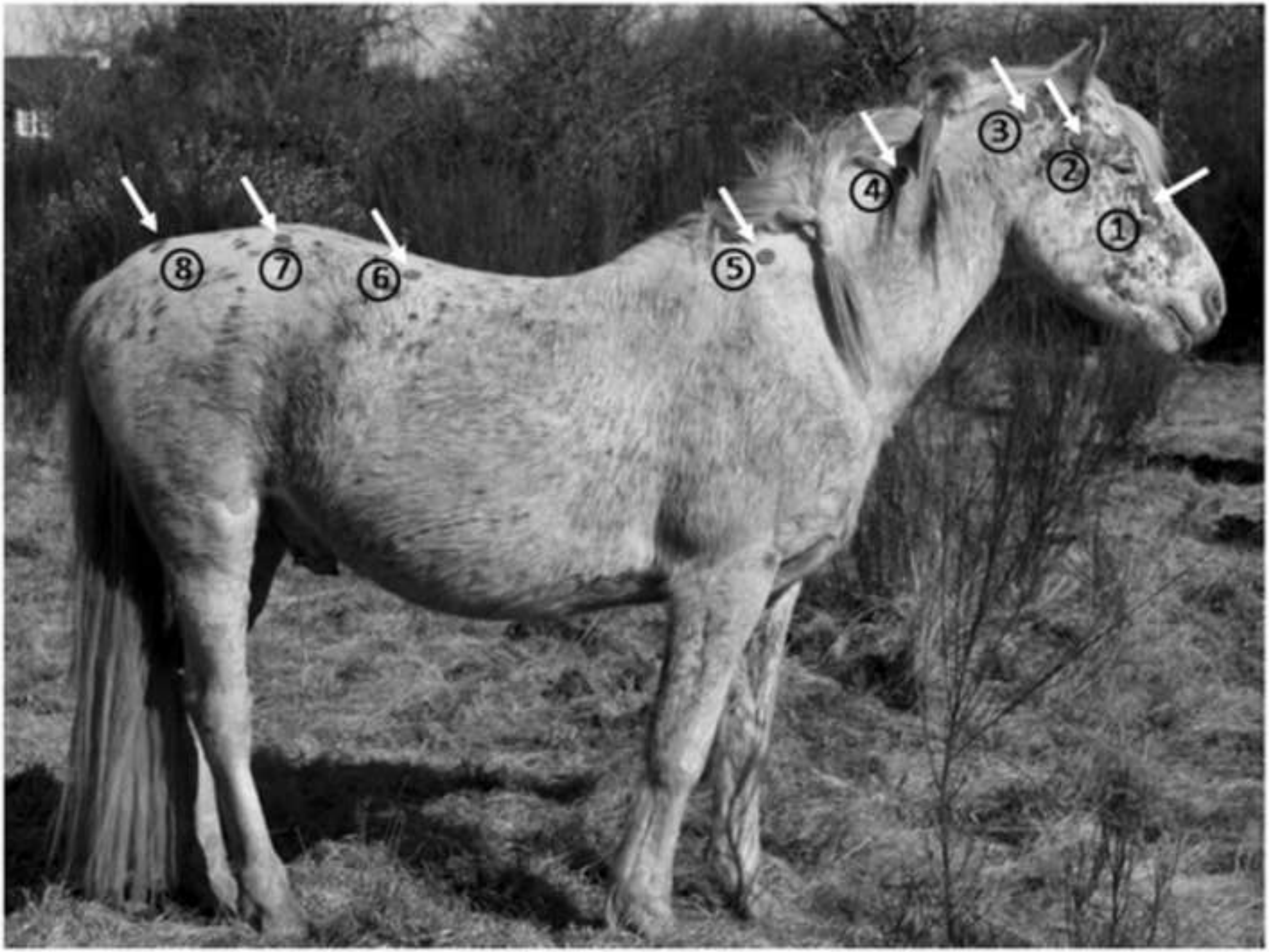

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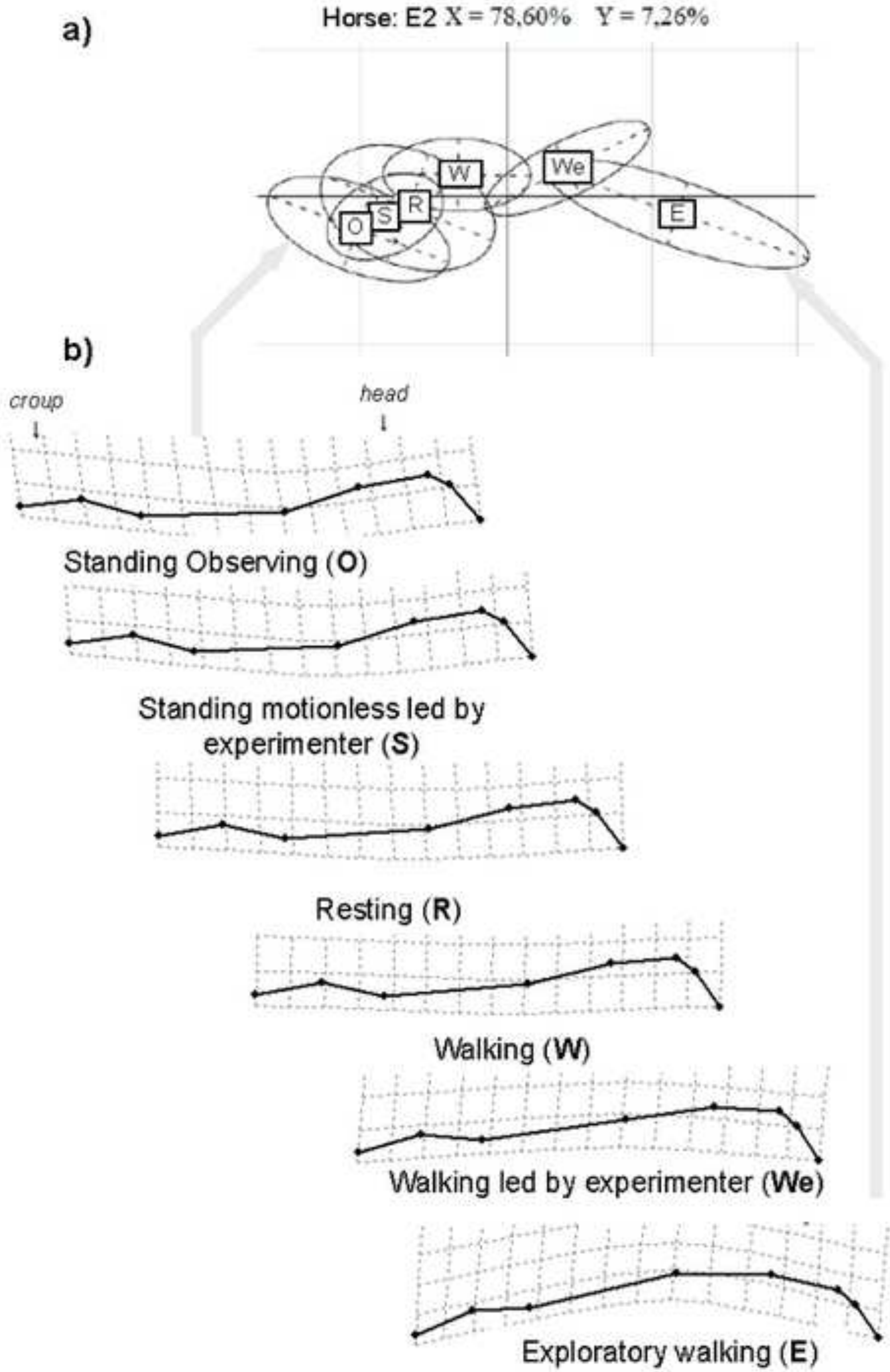

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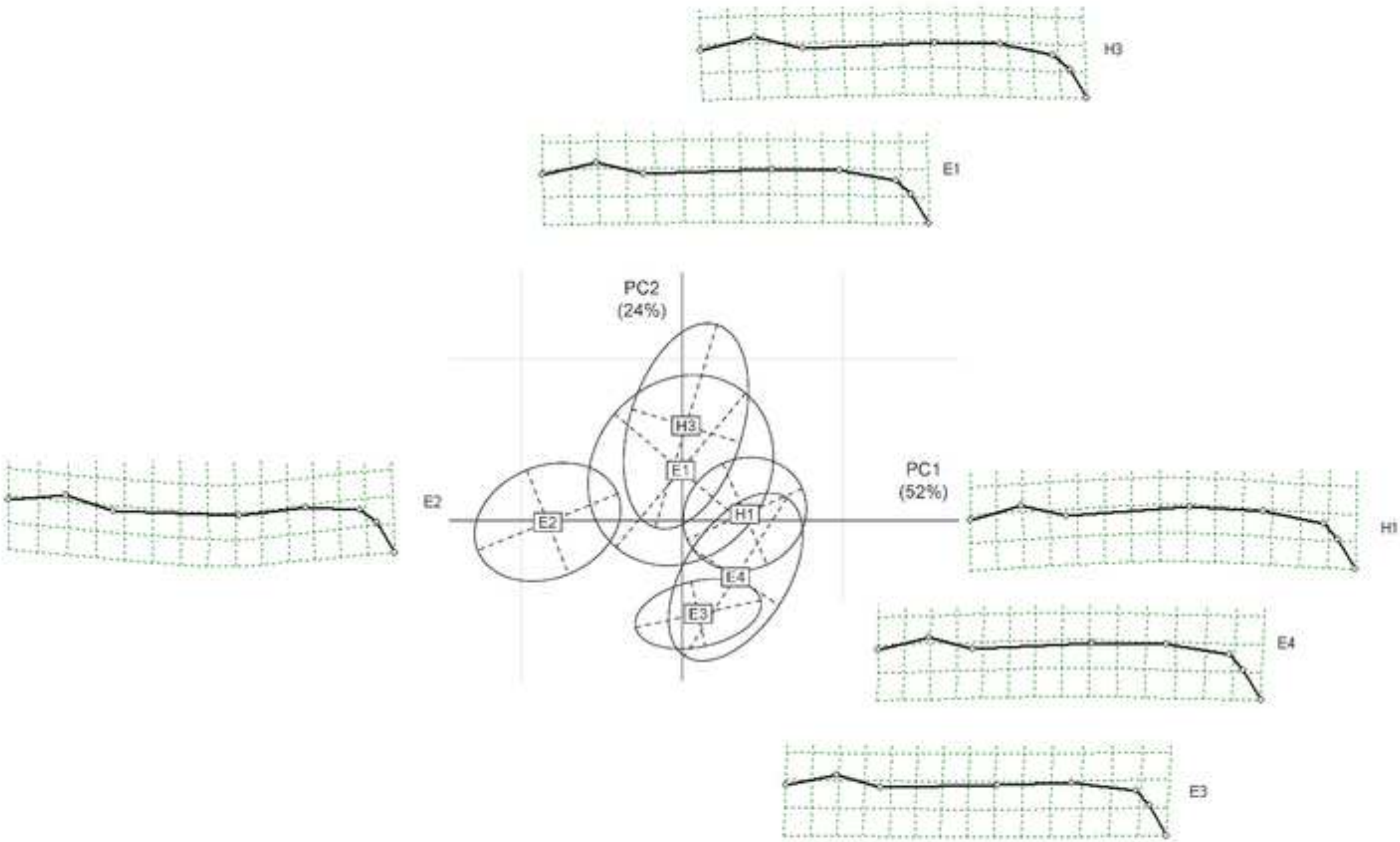

Figure4

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