

## **Introduction**

There is vast diversity in primate external eye pigmentation patterns but no current explanation for this diversity receives ample consensus. Most hypotheses propose that eye color patterns function in intra or inter-specific communication (Sánchez-Villagra & van Schaik, 2019; Mearing et al., 2022; Waciewicz et al., 2022), and that variation in species-specific selection for conspicuous or cryptic eyes, lead to variations in patterns of coloration (Kobayashi & Kohshima, 2001). Among proponents of communicative functions, most attention has gone into the proposal that conspicuous eyeballs afford gaze-following by conspecifics (Kobayashi & Kohshima, 2001; Perea-García et al., 2019; Mearing et al., 2022; Caspar et al., 2021; Kano et al., 2022; Clark et al., 2023). Another proposal about communicative functions of ocular pigmentation is the “gaze camouflage” hypothesis (Kobayashi & Kohshima, 2001), which proposes that cryptic eyes hamper the perception of eye contact, thus reducing instances of physical aggression triggered by eye contact between conspecifics. Indeed, expert ratings suggest that the variation in tolerance to eye contact within primates is proportional to how egalitarian the dominance style of the species is (Harrod et al., 2020), suggesting that subtle changes in external eye appearance facilitate or hinder the maintenance of eye contact rather than eye-gaze following. The last communicative hypothesis is the “self-domestication” hypothesis. It proposes that reduced conjunctivo-scleral pigmentation is the result of pleiotropic effects of selection against conspecific aggression (Perea-García et al., 2019; Mearing et al., 2022; Wilkins et al., 2014) - because melanocytes and the cells of the adrenal medulla derive from neural-crest cells, selecting for reduced emotional reactivity (e.g. hypofunction of the adrenal medulla; delayed migration of neural-crest cell-derived populations) also indirectly selects for reduced melanin synthesis (Wilkins et al., 2014). Therefore, eye depigmentation, while initially a by-product, may become an honest signal of the animals’ temperament (Sánchez-Villagra & van Schaik, 2019).

A few other studies also investigate the proposal that eye color is primarily serving a photo-regulatory function, and differences in eye color primarily reflect differences in habitat lighting, such as the amount of UV radiation in a species’ habitat (Perea-García et al., 2021; 2022). Kobayashi & Kohshima (2001) briefly considered photo-regulation as a driver of variation in conjunctivo-scleral pigmentation in primates, but they dismissed this possibility because of the pigmentation that is apparent around the irises of nocturnal monkeys in their sample (*Aotus spp.*). Perea-García et al. (2021) pointed out that the asymmetric ocular coloration patterns of macaques suggested photo-regulatory functions. Perea-García et al. (2022) later supported this hypothesis by showing that conjunctival pigmentation diminishes with distance from the equator, similar to skin pigmentation in humans (Jablonski & Chaplin, 2010). Their results showed that irises reflect more blue light with increasing distance from the equator, suggesting that iridal coloration made up for lack of blue light in septentrional latitudes - again, resembling patterns observed in humans (Workman et al., 2018).

Finally, developmental evidence (Perea-García, 2016; Perea-García et al., 2019; Clark et al., 2023) and comparisons between sexes of the same species (Perea-García et al., 2016) suggest that developmental constraints, and perhaps endocrinological differences, play a role in explaining patterns of color variation within species.

Presenting wide variation in external eye appearance (Figure 1), macaques are the most widespread anthropoid primate genus (after humans; Rowe & Myers, 2016) and the best described in terms of social structures, making this genus an ideal model to test hypotheses about the ecological and social drivers of eye color variation. Together with an outgroup species (*Papio anubis*), we digitally quantify the eyes of nine macaque species. We use these measurements to test hypotheses about communicative and ecological functions driving eye coloration.

[FIGURE 1 GOES HERE]

One of the concepts that garners greater consensus in the study of macaques is the notion that different macaque species can be classified in terms of “social grade” (Thierry et al., 2008). Social grades are patterns of co-variation in variables related to the social structure of a species, based on a scale from one (most despotic) to four (most tolerant). Social grades are based on observations of interactions between females because females are at the center of social groups in macaques, as they remain in their natal group throughout their lives. A social grade of one denotes a species with steep dominance hierarchy, uni-directional patterns of aggression from more dominant to more submissive individuals, and little to no counter-aggression or reconciliation among non-kin. At the other extreme, grade four species are characterized by shallower dominance hierarchies, less skewed aggression, and greater reconciliation among non-kin following conflict. The notion that macaques have generally poor tolerance for eye contact comes from the extensive study of rhesus macaques (*M. mulatta*) (Gómez, 1996; Emery, 2000), which is a grade 1 species (Thierry et al., 2008). According to the “gaze camouflage” hypothesis, more intolerant (i.e., grade 1) species should have less conspicuous eye morphology to avoid triggering instances of physical aggression. More tolerant (i.e., grade 4) species could have less cryptic morphologies, because the baseline risk of escalated conflicts is lower. Thus, here we test whether there is a link between irido-scleral and pupillo-iridal contrast, and social style grade in female macaques.

It is also possible that male-male interactions drive variation in external eye appearance in macaques. Male macaques in troops containing males and females may engage in coalitionary aggression to compete for rank or access mates (Bissonnette et al., 2014). We investigate a potential link between male ocular conspicuity and the frequency with which males of the species in our sample engage in male-male coalitionary aggression.

The widespread distribution of macaques makes them ideal to test the photo-regulatory hypothesis for eye color diversity. Recent evidence suggests that primate eye morphology is related to environmental photopic factors (Perea-García et al., 2022). For example, iris color shifts towards bluer hues farther away from the equator, suggesting that iris color helps primates regulate their circadian rhythm by maximizing blue light reaching intrinsically photosensitive retinal ganglion cells (Do & Yau, 2010). Further, Zhang & Watanabe (2007) provided preliminary evidence that in Japanese macaques (*M. fuscata*), animals living on islands to the north tended to have more green-blue eyes compared to animals living on southern islands (who had more

yellow eyes). Here, we test whether macaque irises become bluer farther from the equator. We also test whether latitude is related to levels of pigmentation of facial skin, sclera, and conjunctiva between species, as conjunctival pigmentation was found to decrease with distance from the equator (Perea-García et al., 2022). Previously, temporal-nasal asymmetries in the pigmentation of each eyeball in macaques were pointed out as suggestive of photo-regulatory functions (Perea-García et al., 2021). For example, a more pigmented temporal quadrant would absorb the higher amount of UV-light that is present in that side of the face, before it is beamed to the nasal side (Coroneo et al., 1991). We quantitatively compare individual differences in conjunctival pigmentation between the temporal and nasal regions of the eyeball to test whether exposure to sunlight increases pigmentation levels, that would suggest photo-regulatory functions (Perea-García et al., 2021; 2022).

Lastly, studies inspecting primate eye morphology showed that there are within-species differences between sexes and age groups in levels of conjunctival (humans: Russell et al., 2014; orangutans: Perea-García, 2016; chimpanzees and bonobos: Perea-García et al., 2019; chimpanzees: Clark et al., 2023) and iridal pigmentation (chimpanzees and bonobos: Perea-García et al., 2019). Here, we compare our measurements of external eye appearance between age groups and sexes and speculate in terms of putative mechanisms underlying variation in pigmentation as well as developmental trajectories (Supplementary Materials).

## **Materials and methods**

### *Samples*

Photographs were taken on-site by authors of the study (n=1121). These photos were pre-selected from a much larger pool based on whether the different parts of the eye were visible. We selected photos that were good enough quality, that were not obviously under- or overexposed. Our samples included the following species of the genus *Macaca* (n=10): *M. assamensis*, *M. fuscata*, *M. leonina*, *M. maura*, *M. mulatta*, *M. nemestrina*, *M. nigra*, *M. radiata*, *M. silenus*, *M. sylvanus*. As an outgroup, we also included photographs of *Papio anubis*, for which we only included photographs of adults. Two of our macaque species were represented by more than one group: *M. fuscata* (three populations) and *M. leonina* (three populations). Of the three populations of *M. fuscata*, one has been translocated from Japan (Minoo) to Austria (Affenberg). For this population, we used the latitude of their population of origin. When multiple photographs were available for an individual, the average measurements of those photographs were used as an individual data point. This resulted in an average of ~101 photographs per species. This number is well above the 12-14 samples per species recommended by Laitly et al. (2021) to obtain reliable color measurements from uncalibrated pictures.

### *Measurements*

We sampled the eye on the left of each photograph, unless it was not visible or was hard to sample due to stark shadows, specular reflections, or other confounders. We used the PAT-GEOM plugin for ImageJ (Chan et al., 2019) to measure brightness (in HSV) of the pupil iris, conjunctiva, sclera, and skin. To do the measurements, we extended a rectangle selection within the pupil for pupil measurements and noted the average brightness value for that selection. For iris measurements, this extended from the edge of the iris and pupil to the edge into the iris and conjunctiva (avoiding e.g. limbal rings). For conjunctiva measurements the

rectangle extended from the outer edge of the iris to the sclera, if visible. Otherwise, we selected the portion of the eyeball with the most visible conjunctiva. Lastly, we used a rectangle to measure the sclera, which we identified as a noticeably depigmented portion of the eyeball most distal from the iris. The height of this rectangle was up to 10% of the iris diameter. The selection terminated whenever the rectangle reached a confounding factor (reflections or shadows), or if pigmentation changed abruptly (indicating a transition from the conjunctiva to the sclera). For skin measurements, we sampled a rectangle immediately below the eye, of the same width and height as the eye, avoiding confounding factors (typically fur, specular reflections, or shadows). To compare nasal and temporal regions of eyeballs, we selected individuals with photographs in which nasal and temporal regions of the eyeball were visibly different and in similar lighting. We measured the temporal region starting from the edge of the iris into the conjunctiva or sclera until pigmentation changed abruptly (indicating the end of the conjunctiva). We selected the same length of the nasal region of the eyeball (or until reaching confounding factors). We used measurements of iris and conjunctiva brightness to calculate the difference in absolute measurements of brightness (Perea-García et al., 2022) for each eye because of the issues Caspar et al. (2021) and Mearing & Koops (2021) pointed out regarding ratio-based measurements (like RIL). We also calculated the difference between measurements of brightness of the pupil and iris. We converted our hue measurements into RGB for ease of interpretation. All data are available in the supplementary materials.

#### *Phylogenetic data*

We used a consensus tree built with molecular data from the 10kTrees project (<https://10ktrees.nunn-lab.org/>) to account for phylogeny in our PGLS. The tree is accessible from the repository for this study.

#### *Statistical analyses*

We used phylogenetic generalized least squares (PGLS). This method incorporates phylogenetic relatedness to account for non-independence of data points between the species in our sample (Felsenstein, 1985). Measurements of Skin, Conjunctival, Iridal, and Scleral Brightness; Iris Hue, and differences in brightness between pupil and iris, and iris and conjunctiva were response variables in separate regression models (PGLS). Latitudes of each population were noted down and used as a predictor in tests of photoprotection. We used Latitude instead of UV index because it is a poor predictor of actual incidence of UV radiation in the eyes (Hatsusaka et al., 2021). Species level conspecific lethal aggression data were obtained from Gómez et al. (2016) and Gómez et al. (2021) respectively, and were used in tests of the self-domestication hypothesis. We noted down the social grade classification for each species from Thierry et al. (2008). We used social grade 2 for *M. assamensis* instead of social grade 3, as OS considered this more accurate. Data on frequency of male-male coalitionary aggression was taken from Bissonnette et al. (2014). These were used to test hypotheses about gaze camouflaging. For the latitude data, we made the values absolute to express distance from the equator. We examined diagnostic plots and applied Tukey Transformation when residuals were not normally distributed, or to improve homoscedasticity. Phylogenetic analyses vary in the number of species and individuals, depending on available data for each variable and species. Variables at the population level are summarized in the Supplementary Materials (Table S1). Analyses conducted in R (4.0.3). We used the `gls` function with Pagel's *lambda* correlation structure (Pagel, 1999) from the `nlme` (Pinheiro et al., 2021) and `ape` (Paradis & Schliep, 2019) package in R for our PGLS analyses.

## **Results**

### **Tests of the gaze-camouflage hypothesis**

We ran two phylogenetic generalized least squares (PGLS) with social style grade at the species level as the predicting variable in the adult females in our sample ( $n=587$ ). In the first PGLS, we used the difference in measurements of brightness between iris and surrounding tissues (Perea-García et al., 2022) as the outcome variable and found no relationship (Intercept: 3.00,  $\beta=0.057$ ,  $SE=0.284$ ,  $t=0.199$ ,  $p=0.842$ ). In the second PGLS, we used the difference in measurements of brightness between the iris and pupil as the outcome variable and found no effect of social grade (Intercept: 6.138,  $\beta=-0.41$ ,  $SE=0.345$ ,  $t=-1.187$ ,  $p=0.236$ ; Figure 2). We ran two separate PGLS with the same outcome variables but frequency of male-male coalitionary aggression as the predicting variable, and using only the adult males in our sample ( $n=502$ ). Males of the different species covered the whole range of frequency of coalitionary aggression, including species like *M. leonina* and *M. nemestrina*, in which it was *never observed*, and species like *M. sylvanus* and *M. assamensis*, in which they are *routinely observed in all or most males* (coded 0 and 3 respectively in Bissonnette et al., 2014). The first PGLS found no effect of frequency of male-male coalitionary aggression on the (Tukey-transformed) difference in measurements of brightness between iris and surrounding tissues (Intercept: 2.904,  $\beta=-0.014$ ,  $SE=0.249$ ,  $t=0.055$ ,  $p=0.956$ ). The second PGLS also found no effect of male-male aggression on the difference in measurements of brightness between iris and pupil (Intercept: 23.929,  $\beta=-2.649$ ,  $SE=2.748$ ,  $t=-0.964$ ,  $p=0.336$ ; Figure 3).

[FIGURE 2 GOES HERE]

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### **Tests of the self-domestication hypothesis**

In the first test, we used the conspecific lethal aggression data used by Mearing et al. (2022), originally from Gómez et al. (2016). These data include a percentage of deaths due to conspecifics at the species level, and were available for all species in our sample, going from 0 to 3.47%. There was no effect of conspecific killing on (Tukey-transformed) conjunctival brightness (Intercept: 1.933,  $\beta=-0.048$ ,  $SE=0.065$ ,  $t=-0.737$ ,  $p=0.462$ ). Because these data include many instances of infanticide, they may not be the most appropriate to use as a proxy of temperament. When using the data from Gómez et al. (2021), focused on adulticide, there was a small significant effect in the opposite direction to the expected, with adulticidal species displaying slightly brighter conjunctivae (Intercept: 1.97,  $\beta=-0.231$ ,  $SE=0.107$ ,  $t=-2.15$ ,  $p=0.032$ ; Figure 4). We found, thus, no support for selection against aggression leading to lighter conjunctiva.

[FIGURE 4 GOES HERE]

### **Tests of photo-regulatory functions**

Irises did not appear bluer with distance from the equator (Intercept: 0.219,  $\beta=0.001$ ,  $SE=0.001$ ,  $t=0.652$ ,  $p=0.514$ ; Figure 5). There was a significant relationship between distance from the equator and measurements of skin brightness (Intercept: 38.125,  $\beta=1.072$ ,  $SE=0.267$ ,  $t=4.009$ ,

$p < 0.01$ ; Figure 6a), while there was no relationship between distance from the equator and measurements of (Tukey-transformed) conjunctival brightness (Intercept:  $-0.001$ ,  $\beta = 0.004$ ,  $SE = 0.002$ ,  $t = -0.362$ ,  $p = 0.718$ ; Figure 6b). Finally, scleral brightness significantly increased with distance from the equator (Intercept:  $27.583$ ,  $\beta = 0.987$ ,  $SE = 0.455$ ,  $t = 2.167$ ,  $p = 0.031$ ; Figure 6c).

[FIGURE 5 GOES HERE]

[FIGURE 6 GOES HERE]

We measured the nasal and temporal quadrants of the conjunctiva in photographs where both eyes of the same animal were clearly visible, the animal was facing the camera, and the animal was looking sideways ( $n = 115$ ). This allowed us to formally test whether, as proposed in Perea-García et al. (2021), the temporal quadrant is consistently more pigmented (i.e., darker) than the nasal. The temporal quadrant was consistently darker than the nasal quadrant (Intercept:  $5.196$ ,  $\beta = -0.533$ ,  $SE = 0.101$ ,  $F = -5.268$ ,  $p < 0.01$ ; Figure 7).

[FIGURE 7 GOES HERE]

## **Discussion**

We used digital measurements of different parts of the eye of a large number of adult individuals from fourteen populations of nine species of macaques, and an outgroup species (*P. anubis*) to test hypotheses about communication and photo-regulatory functions as drivers of eye coloration. We also compared within-species differences between sexes, and between subadults and adults, to add to a growing number of descriptions of external eye appearance across primate species (Supplementary materials). There is no relationship between our measures of irido-conjunctival or pupillary-iridal contrast and social style grade or frequency of male-male coalitionary aggression, which we used as proxies of communicative functions. These results undermine the prediction of the “gaze camouflage” hypothesis that cryptic patterns of pigmentation are adaptive by reducing instances of physical aggression triggered by the perception of eye contact. Previous work showed no relationship between irido-conjunctival contrast and canine size dimorphism (Perea-García et al., 2022) - a reliable proxy of male intrasexual competition (Leutenegger & Kelly, 1977), a context in which staring is expected to take place as a way to establish dominance (Gómez, 1996). Our results suggest that cryptic eye morphologies with low irido-conjunctival or irido-pupillary contrast are not related to overall levels of intraspecific aggression, generalizing the results from Perea-García et al. (2022) to social interactions that extend beyond male intrasexual competition. Inspection of Figure 2 revealed that conspicuity in grades 1 and 4 is greater than in grades 2 and 3, suggesting an intriguing possibility: that the basal state of macaque external eye appearance is less conspicuous, but that species with very steep (grade 1) and very flat (grade 4) hierarchies benefit from conspicuous eyeballs. Grade 1 species could benefit from unambiguous staring, as a means to signal dominance (or submission, through averted gaze), avoiding costly aggression. Grade 4 species may employ gazing cues for affiliation or gaze following. Lastly, even though we tested the predictions of the “gaze camouflage” hypothesis, it should be noted that Kobayashi and Kohshima (2001) also considered that hiding gaze from predators could be adaptive. This proposal has been addressed with positive results in at least one primate species

(*Sapajus apella*; Whitham et al., 2022). Thus, while we consider that the literature has accumulated evidence undermining the “camouflage hypothesis” with regards to conspecifics, this is not so with regards to predators. In short, we found no support for the idea that eyeball detectability decreases in more despotic intolerant social structures, but if a conspicuous gaze serves in both highly competitive and cooperative contexts, a non-linear relationship may more adequately describe a putative link between eyeball detectability and social structures, and it could be that camouflaging gaze is adaptive towards predators, rather than conspecifics.

We further explored the hypothesized relationship between selection against aggression and conjunctival depigmentation (Perea-García et al., 2019; Mearing et al., 2022; Wilkins et al., 2014) by using percentage of deaths by conspecifics as a proxy of selection for aggression, but could not replicate Mearing et al.’s (2022) results. With another dataset focused on adulticide (Gómez et al., 2021), our results went against the predictions, with adulticidal species displaying lighter conjunctivae. Our results support Caspar et al.’s (2023) conclusions that conjunctival depigmentation is not a correlate of selection against aggression, but are at odds with Mearing et al.’s (2022) conclusions, who found that peri-iridal pigmentation was darker in species with greater percentage of deaths due to conspecifics. Given that Caspar et al.’s (2023) sampled actually domesticated species and their wild counterparts, we are inclined to agree with Caspar et al.’s (2023) conclusion. Furthermore, assuming an adaptive value to conjunctival pigmentation (Kobayashi & Kohshima, 2001), it is not reasonable to expect detecting differences in conjunctival depigmentation across an ancient radiation of natural species like primates. Over evolutionary time, the pressures favoring individuals with pigment would eventually overcome the initial developmental constraint that connects selection against aggression with a non-adaptive loss of pigmentation (Wilkins et al., 2014). Important methodological issues in Mearing et al.’s (2022) study (small sample size; imprecise measurements that may have included e.g. temporal wedge in the sampling area for conjunctival pigmentation measurements) reinforce our inclination to conclude that conjunctival depigmentation is not a correlate of selection against aggression, and therefore is unlikely to act as an honest indicator of a wild animal’s temperament (Sánchez-Villagra & van Schaik, 2019).

Irises did not appear bluer farther from the equator. These results are not in line with Perea-García et al.’s. (2022), who found that the irises of 76 species of anthropoid primates shifted to green-blue with distance from the equator. The species in our sample represent, to the naked eye, the typical gamut of primate iris colorations - from amber to green-blue. Furthermore, the distribution of the species in our samples comprehends most of the latitudinal variation present in extant primates. It is thus puzzling that the pattern observed by Perea-García et al. (2022) is not replicated here. Zhang and Watanabe (2007) were the first to provide preliminary evidence that the frequency of blue eyes in Japanese macaques increases in more northern populations. It could be that this pattern is more pronounced within *M. fuscata* than it is at the genus level. The lack of support for the circadian rhythm hypothesis (Perea-García et al., 2021; 2022) in our results calls for further investigations, opening the possibility that processes such as sexual selection play a role in determining iris color as has been proposed in humans (Frost, 2006).

The relationship between measurements of skin brightness and latitude suggests that dermal pigmentation in macaques is subject to similar selective pressures as in humans (Jablonski & Chaplin, 2010). Human skin pigmentation has been explained as responding to two clines: first, to protect the skin from UV radiation, as populations near the equator have been selected for increased pigmentation; second, populations with more northern distributions have been selected for decreased pigmentation to enable vitamin D<sub>3</sub> synthesis in low UV-B environments (Jablonski & Chaplin, 2010).

We found a positive relationship between brightness measurements of sclera (but not conjunctiva) and latitude. The strong effect of latitude on skin and scleral pigmentation lends support to the idea that photoprotection drives patterns of pigment distribution in the eyeballs of primates. However, the lack of relationship between conjunctival pigmentation and latitude is hard to explain. It may be that, in macaques, the pressure to protect the eyeball from sunlight is reduced, so that variation in pigmentation is due to other functions (e.g. communication, sexual selection) or that it is under relaxed selection. A relaxation in the need to protect the eyeball from sunlight may come about due to multiple factors - for example, in humans, the lack of lateral epithelial stem cells in the corneal limbus could allow for reduced pigmentation of the eyeball (Perea-García et al., 2021). It would be especially interesting to examine *Macaca nemestrina*, who display the greatest levels of depigmentation in all measurements in our dataset, despite being one of the species living the closest to the equator. *Macaca fuscata* is also interesting in this respect, showing considerable levels of conjunctival pigmentation despite its distribution being farthest from the equator. The case of *M. fuscata* may be due to their inhabiting land with considerable snow cover for months (Enari, 2014). Ground cover is the most important factor determining irradiation to the eyeballs, and the most intense reflection from ground cover described is fresh snow (88%; Sliney, 2002). Perhaps taking into consideration the reflectance of the typical ground cover in the habitat of *M. nemestrina* could help explain their unusual eye appearance for an equatorial species. Behavioral differences such as a preference for high vegetation cover or low canopy may also reduce exposure to sunlight, leading to reduced selection pressures. Thus, while ambient light appears to be an important factor contributing to variation in patterns of ocular coloration, this is likely not the only abiotic factor contributing to it.

We found strong within-individual differences in pigmentation between nasal and temporal regions of the eyeball, suggesting that the distribution of pigment in the eyeball responds to the so-called “Coroneo effect” of limbal focussing (Sliney, 2002) as proposed by Perea-García et al. (2021). Because of geometric properties of the face, more scattered light reaches the temporal quadrant of the eyeball compared to the nasal. However, because of the shape of the cornea, this scattered light is focused and beamed towards the nasal quadrant of the eyeball, which can receive up to 20 times the dosage of UV radiation compared to the temporal quadrant (Coroneo et al., 1991). Absorbing as much light as possible on the temporal quadrant will prevent ophthalmelioses on the nasal quadrant. In short, intra-individual variation in peri-iridal pigments appears driven by photo-regulatory pressures.



To conclude, our study did not find that measures of ocular conspicuity (conjunctivo-iridal and pupillo-iridal contrast) were related to proxies of steepness of dominance hierarchy (social style grade) nor intraspecific aggression (male-male coalitionary aggression), failing to support the gaze camouflage hypothesis. We found no evidence in favor of the self-domestication hypothesis when comparing levels of conjunctival depigmentation across species with different measures of conspecific lethal aggression. Our analyses did not show that iris color shifts towards blue farther from the equator, nor that the conjunctiva becomes more lightly pigmented with distance from the equator, suggesting that these parts of the eye are under pressure to comply with functions other than photoprotection, or under relaxed selection. Alternatively, it may be that latitude alone is insufficient and factors such as ground and vegetation cover are important. In contrast, both sclera and skin become more pigmented in animals closer to the equator, suggesting photo-regulatory functions of variation in pigmentation of the eyeball. This concords with our finding that the temporal side of the eyeball, which is typically more exposed to scattered sunlight, is more pigmented than the nasal side of the eyeball in the same individuals. These results add to the investigations supporting a role of ecological, but not communicative functions in driving patterns of eye coloration among anthropoid primates.

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### **Supplementary materials**

[SUPPLEMENTARY MATERIALS ARE UPLOADED IN ANOTHER FILE; temporary link: <https://docs.google.com/document/d/15iRJSiwHa107Lbkyn9xdkzf2ecaO8MDYcotaryPa70c/edit>]

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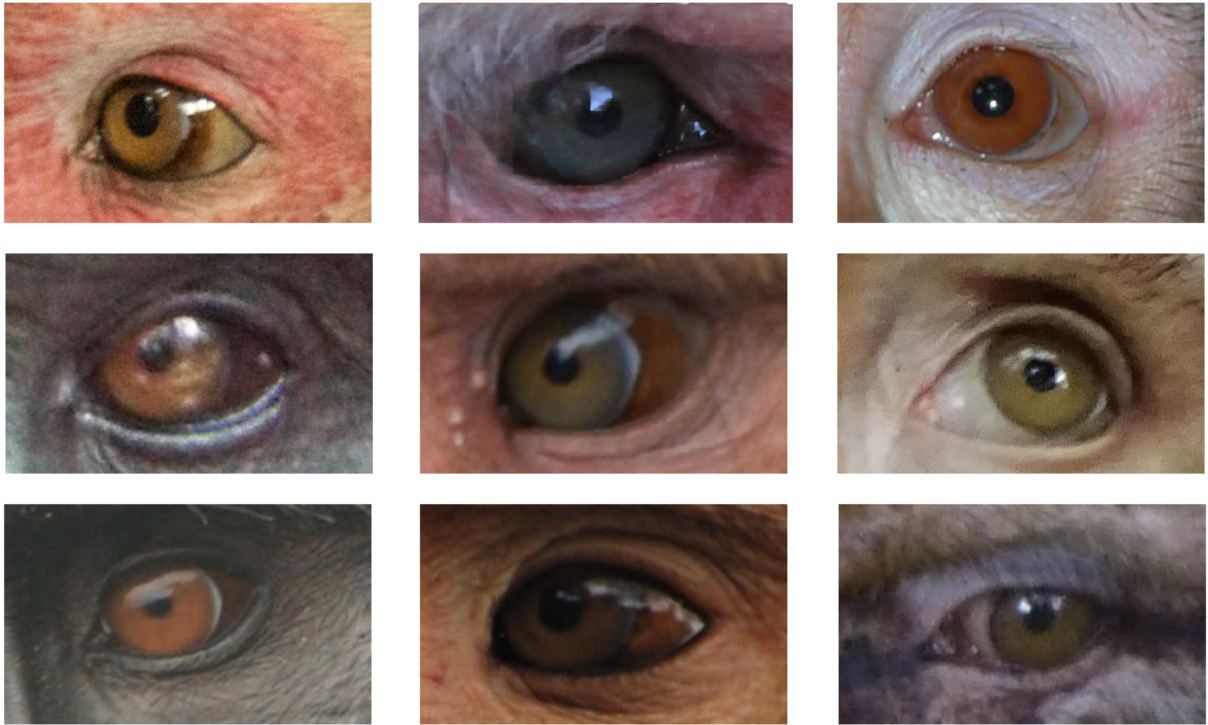


Figure 1.- Macaque external eye appearance is extremely diverse. From left to right and top to bottom: *M. assamensis* (by Julia Ostner and Oliver Schülke), *M. fuscata* (by Alba Castellano-Navarro, Jorg J. M. Massen, Lena S. Pflüger and Pia M. Böhm), *M. leonina* (by Eva Gazagne, Aurélie Albert-Daviaud, and Juan Manuel José Domínguez) *M. maura* (by Víctor Beltrán-Francés), *M. mulatta* (by Stefano Kaburu), *M. nemestrina* (by Nadine Ruppert), *M. nigra* (by Jérôme Micheletta), *M. radiata* (by Shreejata Gupta), and *M. sylvanus* (by Bonaventura Majolo and Laetitia Marechal).

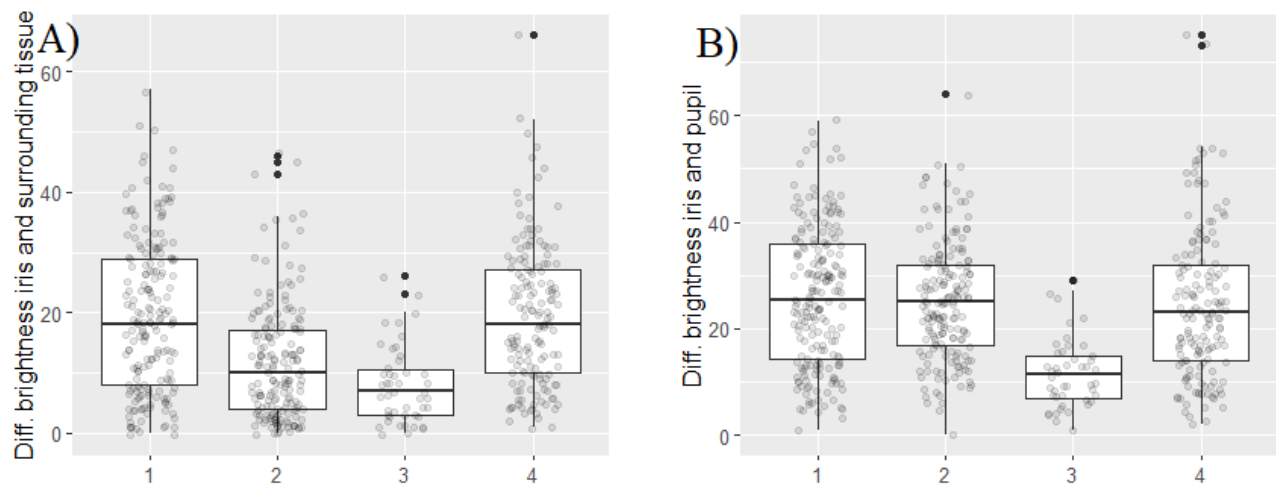


Figure 2.- No relationship between social style grade (X-axis) and A) difference in measures of brightness of the iris and adjacent tissue, or B) difference in measures of brightness of the pupil and iris. These analyses include only measurements from females in our sample. The figure shows raw measurements. The midline shows the median, and lower and upper hinges represent 25th and 75th percentiles, respectively. Black dots represent outliers.

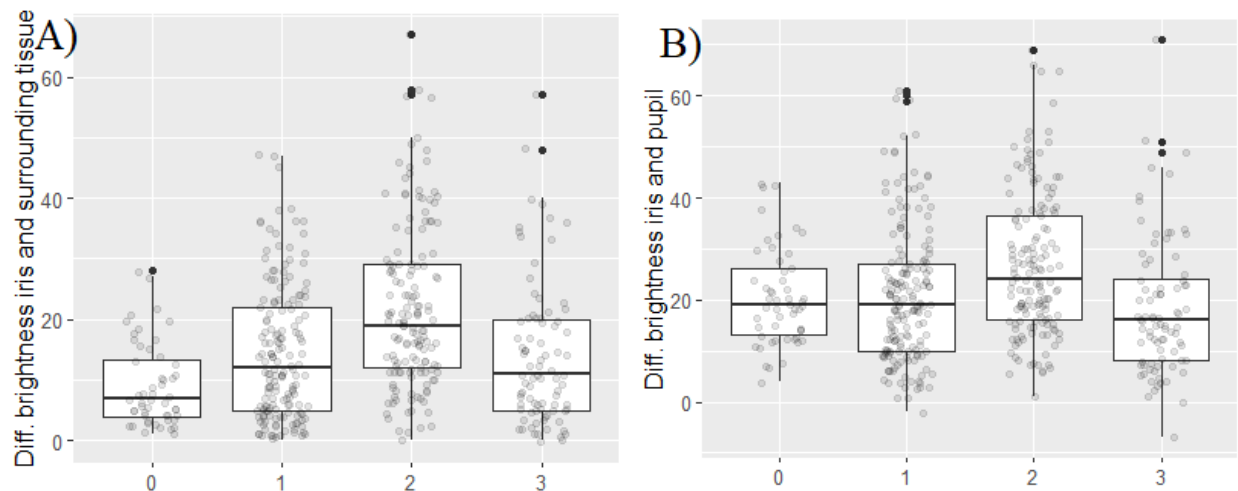


Figure 3.- No relationship between frequency of male-male coalitionary aggression (X-axis) and A) difference in measures of brightness of the iris and adjacent tissue or B) N difference in measures of brightness of the pupil and iris. These analyses include only measurements from

males in our sample. The figure shows raw measurements. The midline shows the median, and lower and upper hinges represent 25th and 75th percentiles, respectively. Black dots represent outliers.

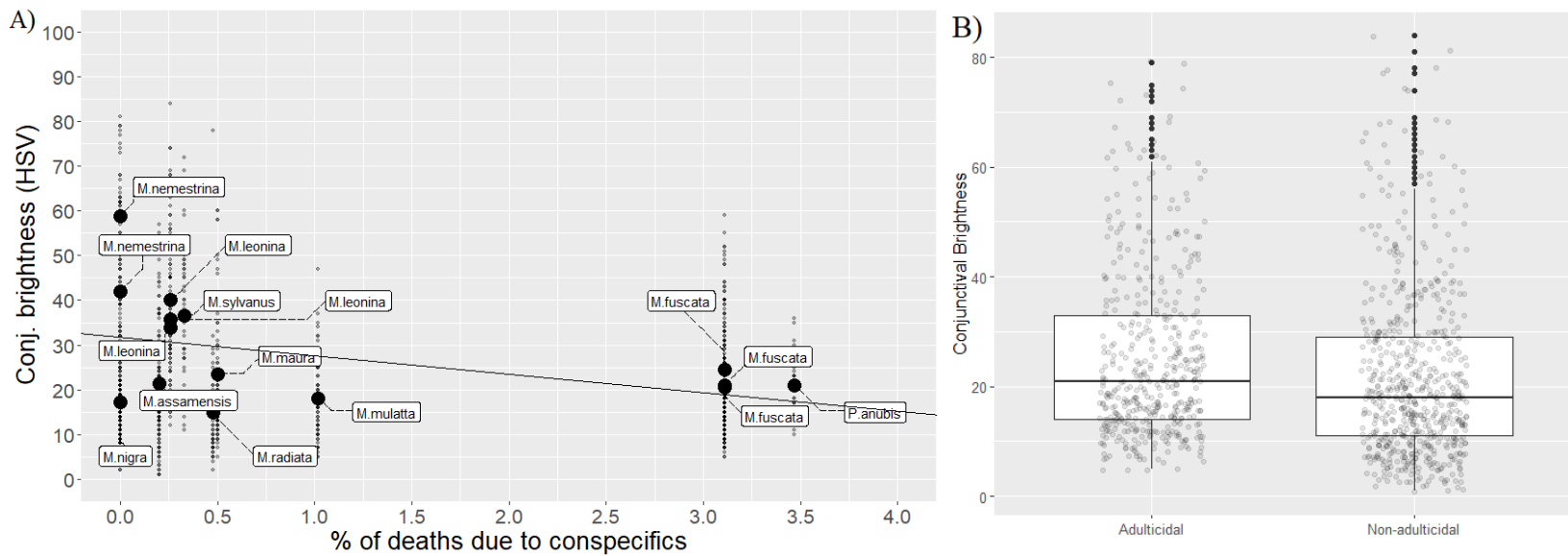


Figure 4.- A) Measurements of conjunctival brightness (HSV) regressed over rates of conspecific killing. Small dots represent individual measurements. Large dots represent population means. B) Measurements of conjunctival brightness depending on whether adulticide has been recorded at the species level. Large dots represent outliers.



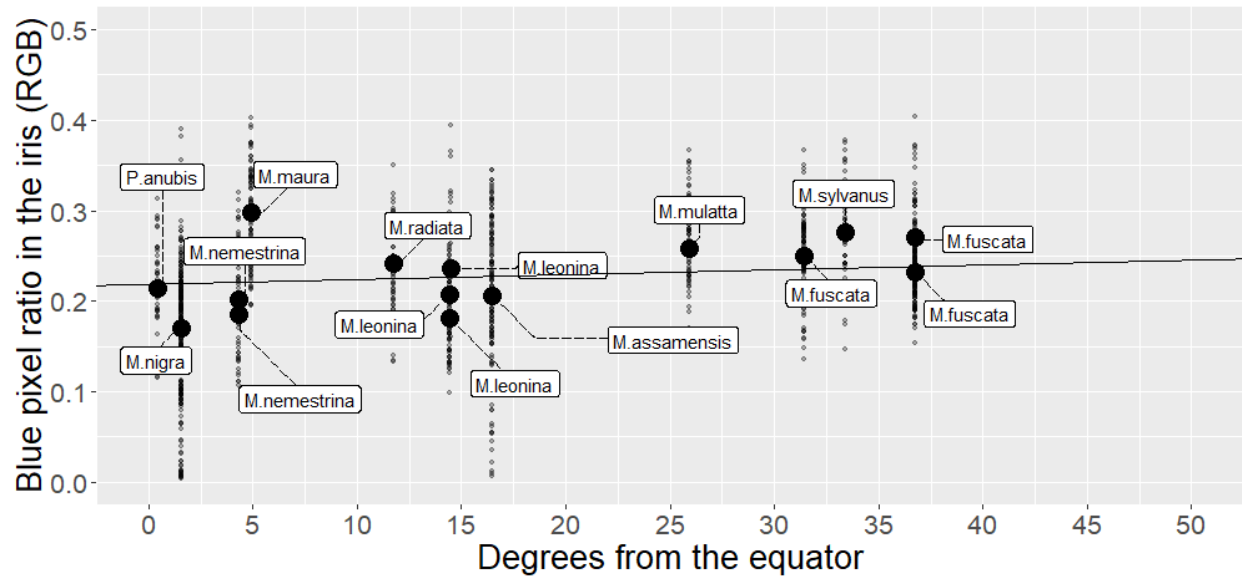
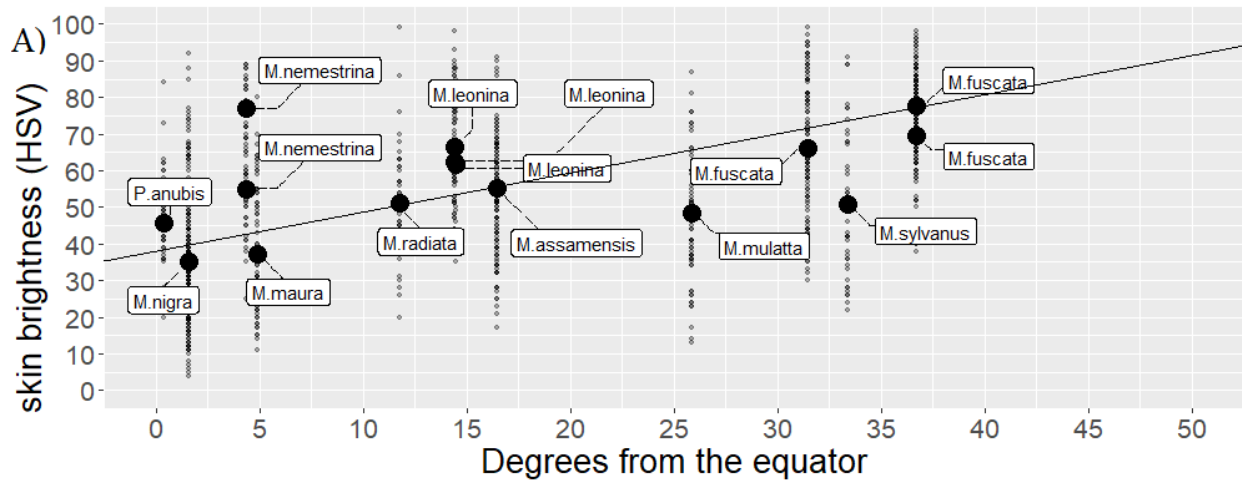


Figure 5.- Irises do not reflect more blue light in species distributed further from the equator.

Small dots represent individual measurements. Large dots represent population means.



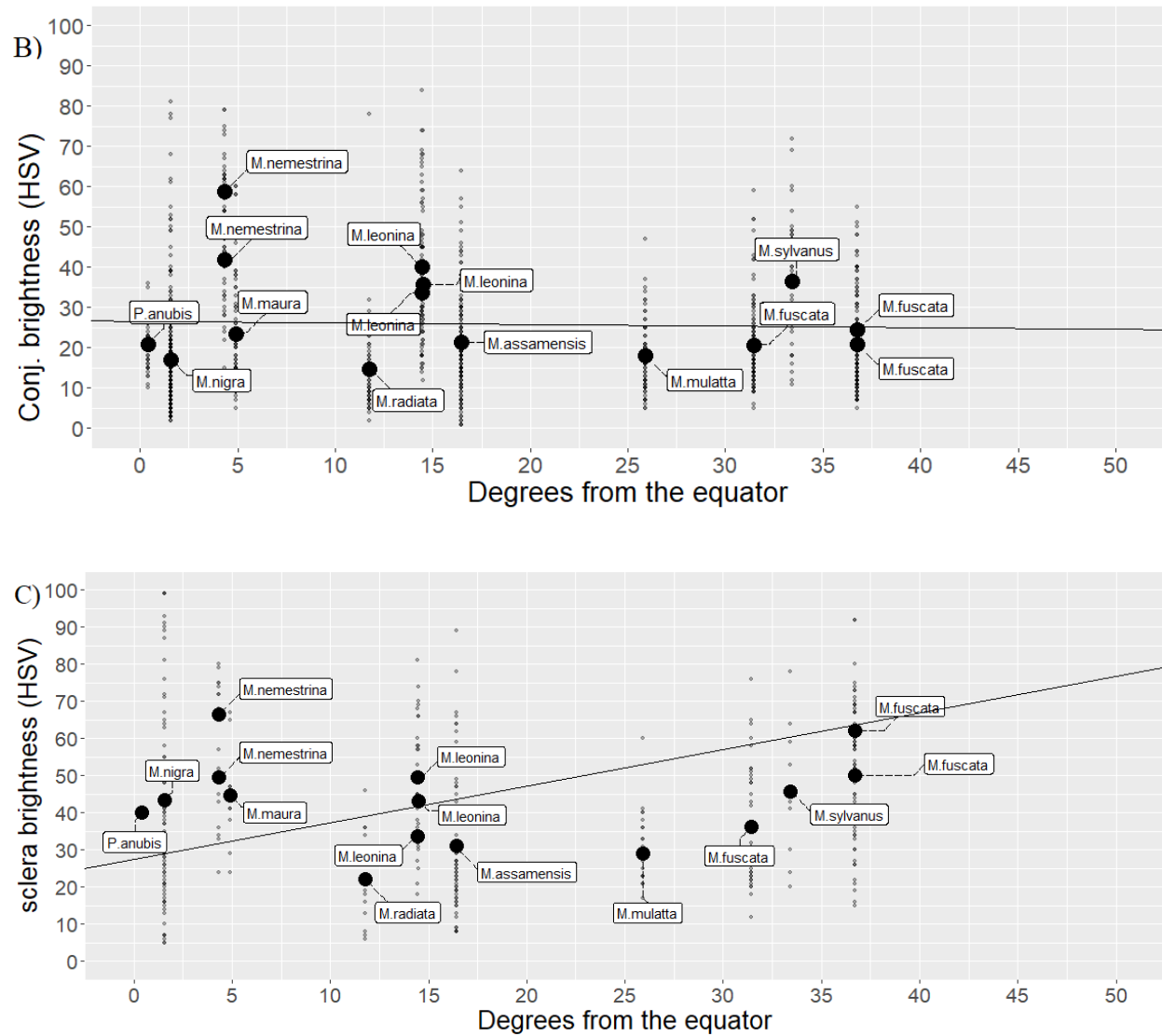


Figure 6.- Measurements of brightness (HSV) of the A) skin, B) conjunctiva, and C) sclera regressed over distance from the equator. Small dots represent individual measurements. Large dots represent population means.

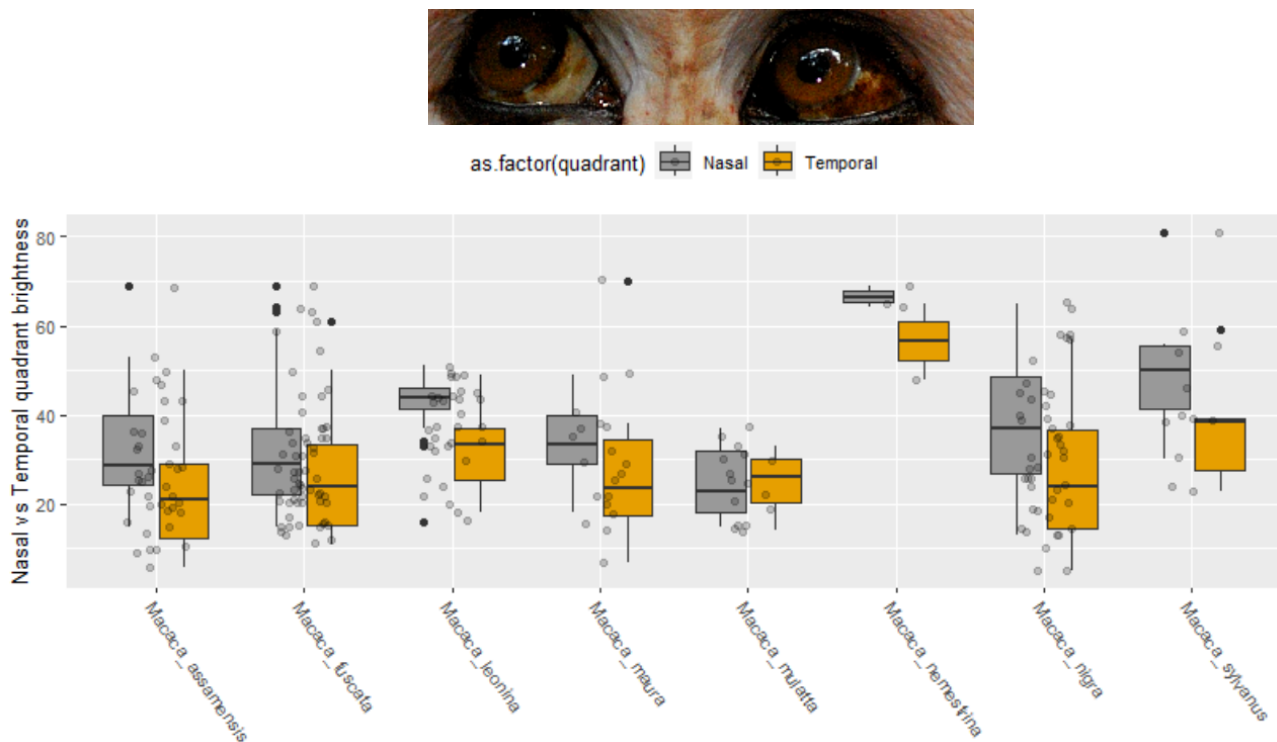


Figure 7.- Measurements of brightness of the nasal (left eye) and temporal (right eye) quadrants of the conjunctiva in the eyes of the same individuals, in the same photograph. The figure shows raw measurements. The midline shows the median, and lower and upper hinges represent 25th and 75th percentiles, respectively. Black dots represent outliers. The eyeballs of a *M. assamensis* are on top, showing the differences between the nasal and temporal quadrants (photo by Julia Ostner and Oliver Schuelke).