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## RESEARCH PAPER

# Effects of Single- and Mixed-Species Group Composition on the Flight Initiation Distances of Plains and Grevy's Zebras

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**Keywords:** flight initiation distance, Grevy's zebras, human approach, mixed-species group, Plains zebras, risk assessment**Abstract**

Zebras, as prey species, attend to the behavior of nearby conspecifics and heterospecifics when making decisions to flee from predators. Plains zebras (*Equus quagga*) and Grevy's zebras (*E. grevyi*) frequently form mixed-species groups in zones where their ranges overlap in Kenya. Although anecdotal observations suggest that Plains zebras are more flighty around humans than Grevy's zebras are, this has not been empirically confirmed, and relatively little is known about how they may influence each other's flight behavior. We addressed these questions by examining the flight initiation distances (FIDs) of Plains and Grevy's zebras in single-species and mixed-species groups from an approaching human. One target individual per group was approached steadily on foot, with start distance, alert distance, and FID recorded from this target. Using start distance and alert distance separately as covariates, 22 Plains zebras in single-species groups exhibited a significantly longer mean FID than 15 Grevy's zebras in single-species groups. The FIDs of 7 Plains zebras and 5 Grevy's zebras tested in mixed-species groups were virtually equivalent and intermediate to those of Plains and Grevy's zebras in single-species groups, suggesting a bidirectional moderating influence of heterospecifics on risk assessment. This effect was most pronounced for Plains zebras in mixed-species groups that exhibited an FID that was significantly shorter than that of Plains zebras in single-species groups. Our findings underscore the importance of recognizing that related equids may be differently impacted by anthropogenic stress.

**Introduction**

Studies of interspecific interactions in mixed-species groups have focused primarily on the adaptive benefits of the presence of heterospecifics on foraging or antipredator behavior (Fitzgibbon 1990; Pays et al. 2014). With antipredator behavior, the dynamics of mixed-species group interactions, such as trade-offs between vigilance and foraging, are similar to those of single-species groups, although details of the processes shaping pre-flight and flight behaviors are much less known (Périquet et al. 2010).

Behavioral study of mixed-species groups has been mostly focused on birds and primates with relatively sparse study of other mammals (Stensland et al. 2003). Vigilance does not seem to be affected

consistently by the presence of heterospecifics; for example, greater kudu (*Tragelaphus strepsiceros*) decreased their vigilance in the presence of heterospecifics (Périquet et al. 2010), but springbok (*Antidorcas marsupialis*) did not (Bednekoff & Ritter 1994). Species differences in responding to social information from conspecifics and heterospecifics might be related to personality factors reflecting a general level of boldness (Kurvers et al. 2010).

Another reason it is important to learn more about how mixed-species groups of animals operate is because climate change is altering ranges and the extent of sympatry for many species. It follows then that if the presence of other species influences predator perception and antipredator responses, then changes in the ecosystem in terms of species

composition due to factors such as climate change may alter a given species' overall response to predators.

The ranges of most present-day equids do not overlap much, with the exceptions of Plains zebra (*Equus quagga*) and Mountain zebra (*E. zebra*) in a small region in southern Africa, Asiatic Wild Asses (*E. hemionus*) and Przewalski's horses (*E. przewalskii*) in Asia (Kaczensky et al. 2008), and Plains zebra and Grevy's zebra (*E. grevyi*) in parts of northern and central Kenya (Faith et al. 2013). The fossil record indicates that this partial sympatry extends back in time to the Middle Pleistocene and perhaps even earlier because Plains and Grevy's zebras are estimated to have diverged 1.5 million years ago (Vilstrup et al. 2013). Due to an increasing aridity, the range of Grevy's zebras is moving south and this species is now experiencing more potential contact with Plains zebras (Cordingley et al. 2009).

Where these partially sympatric zebras graze together, they rarely show interspecific aggression and often stay in a cohesive group when fleeing (Keast 1965). As evidence for attentiveness to interspecific behavior, both species react strongly to the alarm calls of other species (Kitchen et al. 2010; Policht et al. 2011) and Plains zebras appear especially attentive to Masai giraffes (*Masai giraffa*) that have long been identified as useful sentinels (Harrison 1936, p. 280). However, just as Plains zebras are generally more social than Grevy's zebra as a species-typical characteristic, Plains zebras may also be more highly vigilant than Grevy's zebras. Based on lion scat, Plains zebras suffer disproportionately lower lion predation than Grevy's zebras (Rubenstein 2010, p. 243). This greater success in avoiding lions might reflect the male Plains zebra social system that entails high general vigilance, and a rank effect, in that if the dominant animal is calm, all the rest are calm (B. Low, personal communication, 2010).

We focus herein on the antipredator flight behavior of mixed-species groups of Plains and Grevy's zebras toward an approaching human, examining interspecific influences on risk assessment. One key reason for contrasting the flight behavior of Grevy's and Plains zebras is anecdotal observations that Grevy's zebras appear to be less wary of humans than Plains zebras, a property that might characterize an evolutionary history of less consistent predation. East Africa experienced an increase in aridity and savannah grasslands approximately 600 000 years ago (deMenocal 2004). Grevy's zebras are adapted to arid grasslands (Faith et al. 2013), an environment with typically lower prey density that attracts lions (De Boer et al. 2010;

Cain et al. 2011). Water-dependent archaic and modern humans (see Coss & Moore 1990; Reynolds et al. 2011) were similarly constrained in hunting more sparsely distributed game in drier habitats. However, in the cooler and wetter areas of southern Africa, fossil evidence of human hunting and butchering indicates that Cape zebras (*E. capensis*), a species closely related to Plains zebras, were hunted effectively (reviewed by Brubaker & Coss 2015).

To date, there has not been much systematic research on how the two zebra species may differ in their flightiness to humans, or how they might influence each other's antipredator behaviors when in mixed-species groups. The technique of measuring the flight behavior of non-habituated populations to approaching humans has frequently served a dual purpose in research. It can reveal a general pattern of antipredator behavior, especially as opportunities to take data from naturally occurring predation events may be rare and unpredictable (Caro 2005; Donadio & Buskirk 2006; Nyahongo 2008) and provide an index of anthropogenic stress (Tarlow & Blumstein 2007).

#### Experimental Rationale and Predictions

This study is a comparison of Grevy's and Plains zebras' vigilance and flight behavior decisions in response to a standardized human-approach protocol, flight initiation distance (FID) testing. We predict that (1) Plains zebras will have longer flight distances than Grevy's zebras and (2) Grevy's zebras in mixed-species groups with Plains zebras will tend to change their behavior by becoming alert sooner (longer alert distances) and/or fleeing sooner (longer flight distances). It is also possible, although it would seem maladaptive, that Plains zebras might alter their alert and/or flight behaviors in the presence of Grevy's zebras, by alerting and/or fleeing at closer distances. Additionally, the 'many eyes and ears' theory of group formation (Lima 1987) suggests that alertness to possible danger, although not necessarily more rapid flight response, would occur earlier in larger groups.

Research on the relationship between alert response and flight behavior indicates that while the dynamic between the two responses is not always straightforwardly formulaic, there is generally more support for a spatially based system of decision rules. Several species (from diverse taxa) appear to follow a remarkably consistent pattern of flight behavior in which the flight distance ratio is approximately 0.44 of the alert distance (Gulbrandsen et al. 2006). Bearing that in

mind, we will examine the possibility that these two zebra species also follow the approximately 0.44 ratio, but that Plains zebras will tend to show overt alert behavior at relatively longer distances.

## Methods

### Study Species

**Plains Zebra:** Plains zebras are the iconic savannah-dwelling zebras found over a large expanse of sub-Saharan Africa. They are obligate grazers, and need access to grass and water daily (Cain et al. 2011), although because they are non-ruminants they can survive well on coarse low-quality grass and are found in grasslands and wooded savannahs and from sea level to 3500 m (Moehlman 2002). In parts of their range, especially Tanzania and Kenya, they are known for combining with wildebeest and forming vast migratory herds (Sinclair 1985). Their social structure consists of breeding groups (harems or family bands) and bachelor groups, and frequently multiple groups of both types will coalesce to form teeming herds (with or without heterospecifics). According to Rubenstein (2010), aggression between males is frequent and can be fierce and males often harass females, although the dominant stallion of a family band will attempt to protect them. Aggression between females is so rare that a single observed episode warranted a paper reporting it (Fischhoff et al. 2009).

**Grevy's Zebra:** The Grevy's zebra is the largest wild equid, with visually striking narrow stripes and large round ears suggesting especially keen hearing. It is more arid-adapted than the Plains zebra, and only lactating females require daily access to water (Rubenstein 2010). When found in the far northern extent of its range, it has a territorial social structure, in which dominant males claim territories around water sources and females and bachelors are nomadic, with few long-lasting bonds. Mothers and foals are socially bonded until weaning, and multiple females with foals will form groups with the next longest lasting social bonds, although they are said to have more female–female aggression than in Plains zebras (Klingel 1974; Fischhoff et al. 2009). At times, females will form crèches and leave a group of foals while they trek to water. As long as bachelor males do not try to breed on the dominant males' territories, they are allowed to pass through, and as long as they acknowledge the territory-holder's dominance properly in a greeting ritual, he may even socialize in an affiliative manner with them. At times, especially in

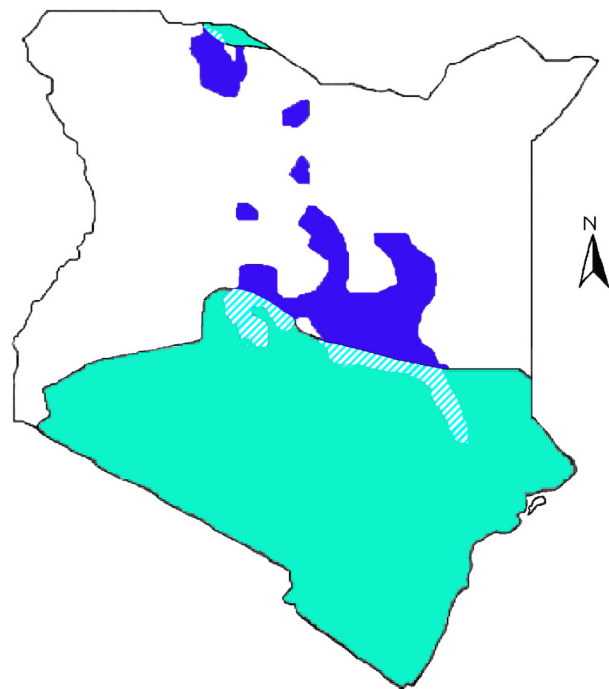
the southern regions, where water is not such a limited resource, Grevy's zebras will group together in temporary large herds, disband, and regroup frequently, which is considered a 'fission–fusion' social structure (Rubenstein 2010).

### Study Sites

To access sympatric Grevy's and Plains zebras, the study was primarily situated in central Kenya (Fig. 1). Data were collected from July–September 2010 mainly at Mpala Research Centre, Laikipia, Kenya, and Ndarakwai Conservancy, Tanzania. Community conservancies to the north of Laikipia in the Samburu district, and Lewa Conservancy near the town of Meru, were also visited. All sites had relatively low density of human beings present (~0.002–0.004 humans/acre) and were areas where hunting was prohibited.

### Experimental Procedures

Flight initiation distance (FID) tests, one of the simplest and most cost-effective methods to quantify animals' fear of humans (Stankowich & Coss 2006; Tarlow & Blumstein 2007; Stankowich 2008), were

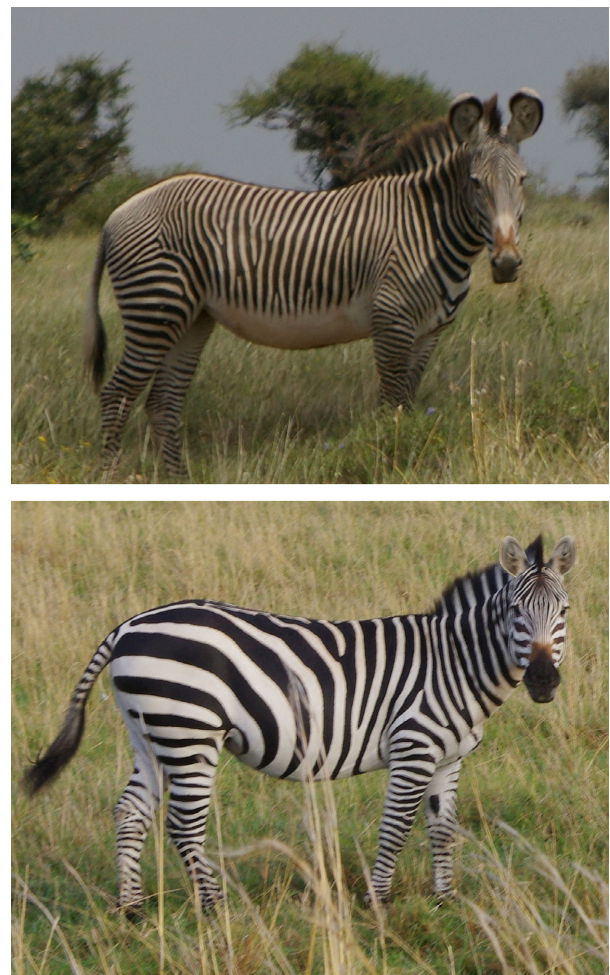


**Fig. 1:** Map of Kenya showing the ranges of Grevy's zebras (blue area) and Plains zebras (green area) with diagonal shading showing the overlapping ranges. Redrawn from Cordingley et al. 2009.

performed by approaching zebras on foot, after locating them by vehicle or by hiking (with the assistance of a local guide). Approaches were only directed to adult 'targets', the closest individual of either sex in the group to the experimenter. We use the generic term 'group' because we approached both smaller groups (bands) and larger groups (herds), which form when many bands coalesce. To minimize the danger of inadvertently sampling a majority of stallions, who tend to position themselves between their band members and any potential threat, the target was established in this quasi-random and objective manner before any band members showed alert behaviors. Once a group was located, the number of zebras present and the group composition (single or mixed species) were noted. Although individuals were not marked, the probability of resampling was very low due to the wide dispersion of groups and the unlikelihood of encountering two different groups with the same combination of adults and juveniles of either or both species. Trials were classified according to (1) which species was targeted and (2) what type of group was being approached (i.e., a single species or mixed species), which yielded four distinct group compositions: Plains-single, Plains-mixed, Grevy's-single, and Grevy's-mixed. To the extent possible in the field, we alternated among the four kinds of group compositions as we conducted trials.

In addition to size and composition of the group being approached, we also recorded in a field notebook (and verbally while videorecording) the proportions of juveniles and adults and proportions of each species (if applicable) in the group, weather, vegetation density, and whether or not humans, other wild-life species, or livestock animals were visible nearby. The total distance to the target individual (start distance) was measured with a laser rangefinder (Leupold RX-1000i, Beaverton, OR) or Bushnell Yardage Pro (Overland Park, KS) and recorded, and the approach (trial) began. The start point was marked by the vehicle, or by leaving a backpack at the starting location. The experimenter (ASB) videorecorded the approaches using a Sanyo (Osaka, Japan) Xacti VPC-CG10 high definition MP4 videorecorder. Dressed in clothing of natural hues, the experimenter walked directly toward the target at a rate of approximately one meter per second (pace was guided by glancing at a digital wristwatch). Hard stares in the animals' direction or sustained direct eye contact with any individual were avoided (see Emery 2000; Bateman & Fleming 2011). When the target zebra exhibited clear alert behavior, the experimenter dropped a weighted flag to mark that location (alert point) and continued

the approach, with as little alteration to the steady pace as possible. Alert behavior was determined when the target ceased grazing (if it had been) and assumed an alert posture with its neck at a higher angle than a typical relaxed standing posture and its head oriented toward the experimenter (Fig. 2), possibly giving alarm barks, snorts, or foot stamps. When the target fled, the experimenter dropped a second weighted flag to mark the location (flight initiation point) and ceased approaching. Flight was defined as deliberate locomotion at any gait, after alertness had been displayed, for two or more steps that served to increase the distance between the zebra and the approaching experimenter. Alert distance and FID were calculated by measuring the distance from the alert point and the flight initiation point, respectively, back to the



**Fig. 2:** Grevy's zebra (top) and Plains zebra exhibiting alert postures. Note the elevated heads and forward-facing ears. Grevy's zebra Photograph by Alexali Brubaker, 2010; Plains zebra photograph by Richard Coss, 2013.

start point, and then subtracting each of those distances from the start distance.

### Ethics Statement

All research procedures were approved by Animal Use and Care Protocols 08-13277 and 16506 from the University of California, Davis, and included under the wildlife research permits issued to the Mpala Research Centre, Laikipia, and the Lewa and Samburu district Community Conservancies, Kenya.

### Video Coding

Videos were reviewed and coded jointly by teams of two trained research assistants, working side-by-side to ensure real-time inter-rater reliability by consensus. We reviewed the videos to confirm the data recorded in the field notebook in real time, especially the counting of group sizes. Vegetation density was categorized from video using an interval scale for three levels. We also quantified the spatial distance between alert distance and FID known as buffer distance (Fernández-Juricic et al. 2001, 2002). If visible, several additional behaviors that sometimes occur after flight were also recorded: the target pausing to monitor the experimenter, joining with group mates in a semicircle or 'fan' and facing the experimenter, and resuming relaxation, inferred by the resumption of behaviors incompatible with vigilance, such as grazing, social behavior, or auto-grooming.

### Data Analysis

Data analyses were performed in JMP (version 10). Conceptually, alert distance can be thought of as a dependent behavioral variable, but it is also established in the literature as a useful predictor of flight initiation, so we analyzed it as both a predictor and a dependent variable. As discussed in detail below, start distances, alert distances, and group sizes were confounded with group composition. These measures were centered using deviations from the means of each of the four group compositions. Centered start and alert distances and group size were then used as distinct covariates in two, two-factor (species and group type) analyses of covariance (ANCOVAs) to partial out their linear relationships with FID and buffer distance. Centered start distance was also used as a covariate in a two-factor ANCOVA examining group differences in alert distance. To examine specific hypotheses, tests of simple effects compared each

species in single- and mixed-species groups. Regression analyses were employed to examine the linear relationships between start distance and alert distance and alert distance and FID. Effect sizes are reported as partial  $\eta^2$  and Cohen's  $d$  derived from equations 2 and 4 in Zakzanis (2001) for comparing the mean differences in groups with unequal numbers of animals. Frequencies of behavioral occurrences were analyzed using Fisher's exact tests. The only other measure among the four zebra groups with reliable data for statistical analysis was the level of vegetation density, the frequency of which was examined using log-linear analysis.

### Results

Overall, we conducted 49 usable trials: 15 Grevy's-single, 22 Plains-single, five Grevy's-mixed, and seven Plains-mixed groups. The small number of mixed-species groups sampled, despite broad searching, reflects the lower density of Grevy's zebras in central Kenya (Rubenstein 2010). Bartlett's test of homogeneity of variances showed that the variances were homogeneous for FID and alert distance for the four groups (FID  $\chi^2_3 = 5.15$ ,  $p = 0.16$ ; alert distance  $\chi^2_3 = 1.37$ ,  $p = 0.71$ ). This homogeneity reduces the probability of a type I error for our moderately unbalanced design (see Tung-Hsing & Olejnik 1996; Wilcox 2002). To examine whether vegetation density level differed among the groups, log-linear analysis with maximum-likelihood estimation showed that the interaction between species, group type, and vegetation density was not statistically significant (likelihood ratio  $\chi^2_2 = 0.002$ ,  $p = 0.99$ ).

Because start distance can influence both alert distance and FID, initial analyses examined whether start distance and alert distance were confounded with group composition. A two-factor (species and group type) analysis of variance (ANOVA) of start distance revealed a statistically significant interaction of species and group type ( $F_{1,45} = 5.63$ ,  $p = 0.022$ ), the major source of which was the mean difference in start distances (Table 1) for Plains and Grevy's zebras in single-species groups (simple effect:  $F_{1,45} = 15.75$ ,  $p < 0.001$ ,  $d = 1.36$ ). The average Plains-single start distance was 94.54 m longer than the average Grevy's-single start distance. Similarly, the mean alert distance of Plains zebras was 72.16 m longer than that of Grevy's zebras in single-species groups (simple effect:  $F_{1,45} = 12.92$ ,  $p < 0.001$ ,  $d = 1.28$ ). In contrast, the Plains and Grevy's zebras in mixed-species groups had start distances that did not differ appreciably (simple effect:  $F_{1,45} = 0.215$ ,  $p = 0.65$ ,  $d = 0.25$ ).

**Table 1:** Behaviors associated with FID tests. Means (and standard deviations) are shown for all values except FID/alert ratio, which shows 95% confidence intervals

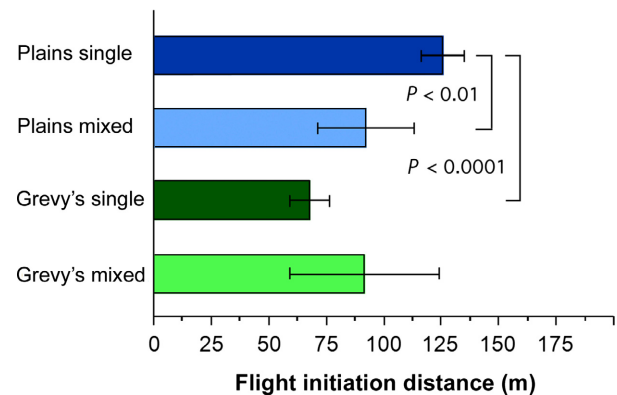
Group composition (N)	Group size	Start distance	Alert distance	FID	Buffer distance	FID/Alert ratio (regression slope)
Plains-single (22)	18.86 (28.97)	188.41 (78.96)	160.96 (60.08)	125.86 (43.60)	35.09 (38.45)	0.76 (0.67–0.80)
Plains-mixed (7)	25.86 (34.92)	117.29 (70.61)	110.57 (71.77)	92.29 (54.87)	18.28 (20.54)	0.81 (0.73–0.89)
Grevy's-single (15)	3.93 (3.15)	93.87 (51.75)	88.80 (50.21)	67.93 (32.92)	20.87 (21.26)	0.73 (0.68–0.83)
Grevy's-mixed (5)	8.20 (3.27)	136.60 (85.77)	114.60 (70.30)	91.80 (72.93)	22.80 (12.91)	0.85 (0.67–1.02)
Overall (49)	14.20 (24.29)	144.02 (80.89)	126.94 (66.41)	99.86 (51.10)	27.08 (30.00)	0.77 (0.72–0.81)

Because Plains zebras frequently aggregate in larger numbers, we examined group size (the number of animals) across group compositions. Indeed, group size was confounded with species for single-species groups (simple effect:  $F_{1,45} = 4.18$ ,  $p = 0.047$ ,  $d = 0.66$ ), with Plains-single groups on average 15 zebras larger than Grevy's-single groups, but no significant difference was found across mixed-species groups nor was there a significant interaction between species and group type.

### Flight Initiation Distance

Initial analysis revealed that centered group size as a covariate in a two-factor (species and group composition) ANCOVA on FID contributed little as a control ( $\beta = 0.11$ ;  $p = 0.48$ ). An ANCOVA model constructed using centered start distance as the covariate to compare FIDs yielded 0.71 proportion of variance explained and revealed a main effect of species ( $F_{1,44} = 9.04$ ,  $p = 0.004$ ) and an interaction between species and group type ( $F_{1,44} = 8.74$ ,  $p = 0.005$ ). Tests of simple effects examining the sources of this interaction detected a reliable species effect across single-species groups (Fig. 3), with the mean FID of Plains zebras in single-species groups significantly longer than the mean FID of Grevy's zebras in single-species groups ( $F_{1,44} = 36.09$ ,  $p < 0.001$ ). This difference in mean FID (57.93 m) exhibited a large effect size ( $d = 1.46$ ). Plains zebras in single-species groups also had a significantly longer mean FID than Plains zebras in mixed-species groups (simple effect:  $F_{1,44} = 7.22$ ,  $p = 0.01$ ) with a mean difference of 33.57 m and medium effect size ( $d = 0.72$ ). The mean FIDs of Grevy's zebras in single- and mixed-species groups did not differ significantly (simple effect:  $F_{1,44} = 2.57$ ,  $p = 0.116$ ,  $d = 0.53$ ). The mean FIDs of Grevy's (91.8 m) and Plains zebras (92.3 m) in mixed-species groups were virtually equivalent (simple effect:  $F_{1,44} = 0.0009$ ,  $p = 0.98$ ,  $d = 0.008$ ).

Another ANCOVA using centered alert distance as the covariate yielded 0.82 proportion of variance explained and resulted in nearly the same pattern of



**Fig. 3:** Flight initiation distances of Plains and Grevy's zebras in single-species and mixed-species groups. Mean and standard error values are shown. Significance levels were derived using ANCOVA with centered start distance as the covariate.

results, with the exception of an additional simple effect: There was a statistically reliable difference ( $\alpha = 0.05$ ) between the FIDs of Grevy's zebras in single-species vs. mixed-species groups ( $F_{1,44} = 4.16$ ;  $p = 0.047$ , mean difference = 23.87 m with medium effect size:  $d = 0.53$ ). This model also revealed a main effect of species averaged for group type ( $F_{1,44} = 14.64$ ,  $p = 0.0004$ ) and an interaction of species and group type ( $F_{1,44} = 14.14$ ,  $p < 0.001$ ) resulting from the large species difference for single-species groups (simple effect:  $F_{1,44} = 58.38$ ,  $p < 0.001$ ,  $d = 1.46$ ). In addition to the aforementioned reliable difference in Grevy's zebras in single-species and mixed-species groups, a third contributor to this interaction was that Plains zebras in single-species groups exhibited a significantly longer FID than Plains zebras in mixed-species groups (simple effect:  $F_{1,44} = 11.67$ ,  $p < 0.005$ ,  $d = 0.72$ ).

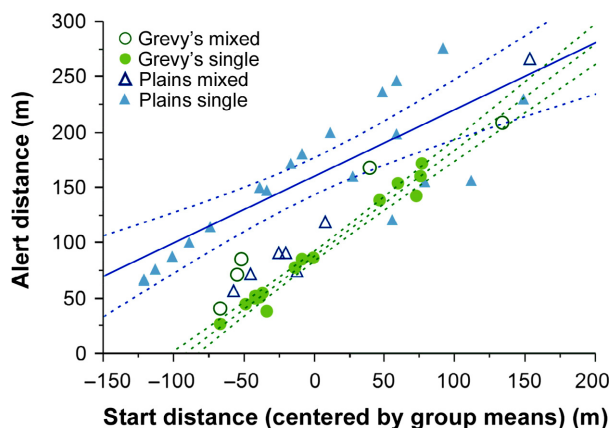
### Alert Distance and Instant Alert

Alert distance is a behavioral response of interest in itself as it indicates awareness of a potential threat and the beginning of a decision process regarding the prey animal's response, as well as for its relationship with

the subsequent flight. Regression of start distance (centered) as the predictor variable for alert distance (Fig. 4) for Plains single-species groups and Grevy's single-species groups revealed strong associations (Plains:  $F_{1,20} = 33.69$ ,  $p < 0.001$ ; Grevy's:  $F_{1,20} = 540.23$ ,  $p < 0.001$ ).

Linear regression further revealed that group size did not predict alert distance reliably (log-transformed;  $R^2 = 0.036$ ,  $F_{1,47} = 1.77$ ,  $p = 0.19$ ), a finding that does not support the 'many eyes and ears' prediction that larger groups would become alert sooner. A related regression analysis using centered values for group size to address the question of a group-size effect, controlling for the fact that the two species differ in average group size, also did not reveal a reliably predictive relationship ( $R^2 = 0.005$ ,  $F_{1,47} = 0.25$ ,  $p = 0.62$ ). Additionally, when either of these regressions was restricted to test one group composition at a time, none of the group compositions showed a group-size effect.

With respect to mean differences in vigilance, an ANCOVA with start distance (centered) as the covariate and alert distance as the dependent variable revealed a significant main effect for species ( $F_{1,44} = 12.00$ ,  $p < 0.005$ ) and a significant interaction of species and group type ( $F_{1,44} = 14.99$ ,  $p < 0.005$ ). This model explained 0.82 proportion of the variance. Analyses of simple effects showed that Plains zebras in single-species groups had a significantly longer mean alert distance (160.96 m) than Grevy's zebras in single-species groups (88.80 m) ( $F_{1,44} = 54.60$ ,  $p < 0.001$ ) with a large effect size ( $d = 1.28$ ). Plains zebras in mixed-species groups had a reliably shorter



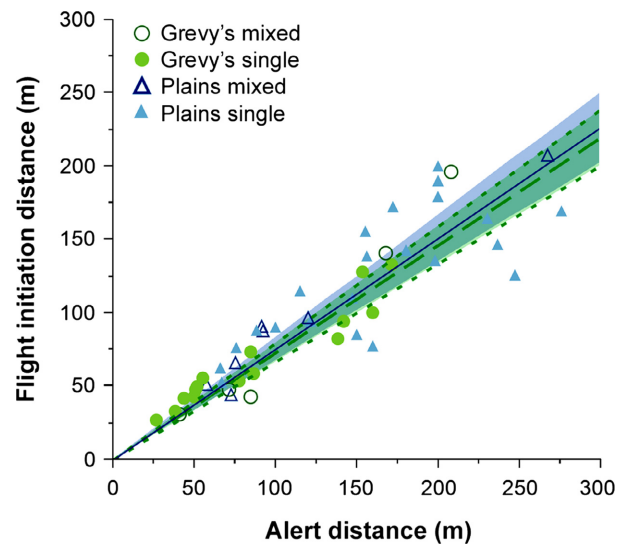
**Fig. 4:** Association of centered start distances and alert distances. Solid slope line represents Plains zebra in single-species groups. Dashed slope line represents Grevy's zebras in single-species groups. 95% confidence intervals are depicted by curved dashed lines. Trends are apparent for Grevy's and Plains zebras in mixed-species groups.

mean alert distance (110.57 m) than Plains zebras in single-species groups (simple effect:  $F_{1,44} = 15.85$ ,  $p < 0.001$ ) also with a large effect size ( $d = 0.80$ ). Conversely, Grevy's zebras in mixed-species groups exhibited an increase in their alert distance compared with their cohorts in single-species groups with a trend toward statistical significance ( $F_{1,44} = 2.93$ ,  $p = 0.094$ ,  $d = 0.51$ ). The alert distances of Plains and Grevy's zebras in mixed-species groups were similar ( $p = 0.81$ ,  $d = 0.06$ , see Table 1 and Fig. 4).

Sometimes a target zebra showed an alert posture simultaneously as the experimenter initiated approach toward the group; these were classified as 'instant alert'. Despite longer average starting distances, Plains zebras in single-species groups exhibited a greater frequency of instant-alert trials (13 of 22 trials) than Grevy's zebras in single groups (5 of 15 trials), but this interaction was not statistically significant (Fisher's exact test statistic = 0.18).

#### FID/Alert Distance Ratio

Zebras in this study displayed an FID to alert distance regression slope of approximately 0.77 for all groups. Neither Plains-single and Grevy's-single groups appeared to vary this ratio substantially as evidenced by overlapping confidence intervals for the ratios when calculated separately for each group composition (Table 1, Fig. 5).



**Fig. 5:** Association of alert distances and flight initiation distances. Plains zebras in single-species groups: slope = 0.76 (solid line). Grevy's zebras in single-species groups: slope = 0.73 (long dashed line). 95% confidence intervals are depicted by shaded areas. Plains and Grevy's zebras in mixed-species groups were used to compute the overall slope of 0.77.



### Buffer Distance

A two-factor (species and group type) ANCOVA examining buffer distance, with centered start distance as the covariate, found neither a species main effect nor the interaction between species and group type to be statistically significant (species:  $p = 0.57$ ; group type:  $p = 0.38$ , species and group-type interaction:  $p = 0.28$ ).

### Fan Behavior

Grevy's zebras in single-species groups were the only group composition observed to perform fan behavior in FID tests. Trials were omitted from this frequency analysis if zebras could not be seen clearly enough on the videorecording to determine whether the group had formed a fan. For the subset of single-species trials with clear visibility after flight, 3 of 13 Grevy's zebra groups fanned, while none of the 14 Plains groups fanned (Fisher's exact test statistic = 0.098).

### Discussion

The primary hypothesis that Plains zebras are generally more flighty than Grevy's zebras was supported, as evidenced by their consistent tendency to flee an approaching human at significantly longer distances. The prediction that Grevy's zebras in mixed-species groups would lengthen their flight distances compared with Grevy's in single-species groups was also supported when alert distance, but not start distance, was controlled statistically. Interestingly, the theoretically less likely prediction that Plains zebras might also modify their behavior by decreasing their flightiness when in mixed-species groups was also supported; Plains zebras targeted for approach in mixed-species groups fled at a shorter average FID than Plains zebras targeted in single-species groups.

It is important to note that Plains zebras in single-species groups showed overt alert behavior at a greater distance than Grevy's zebras in single-species groups even after controlling for their typically longer start distances (see Fig. 4). As operationally defined in this and similar studies, alert distance is not necessarily a perfect indicator for the animal's internal state of awareness of the approaching experimenter. Many prey species have such acute sensory capacities compared to human beings that they are usually aware of humans well before the humans are aware of them. As Fernández-Juricic et al. (2002) point out, it may well be adaptive under certain circumstances for a prey animal to conceal its awareness of the predator

by remaining motionless, or carrying on foraging or other activity, until it becomes expedient for the prey to behaviorally announce its awareness of the predator.

Although it is not clear why Plains zebras in single-species groups showed alert behavior at further distances than Grevy's zebras in single-species groups, we can propose some possibilities: As a highly conserved retinal adaptation, equids exhibit a horizontal strip (visual streak) with high cone density and neural organization facilitating the detection of motion near the horizon (Sandmann et al. 1996). Horses, and presumably zebras, rotate their eyeballs to keep the visual streak horizontal when their heads are down while grazing (Banks et al. 2015). It seems reasonable to argue that, once an approaching agent is detected irrespective of zebra's head orientation, the difference in the alert distances of Plains and Grevy's zebras likely characterizes evolutionary divergence in their pattern of threat assessment. The aforementioned vulnerability of Grevy's zebras to lion predation in central Kenya (Rubenstein 2010) might reflect their long history of relaxed selection from lions and possibly human hunting in arid regions. Conversely, the longer alert distance of Plains zebras to an approaching agent with uncertain intent possibly reflects their more consistent history of lion predation and human hunting [see Brubaker & Coss (2015)].

We can use the fossil and paleoclimatic record to bolster our argument that Plains and Grevy's zebras experienced different histories of lion predation. Both zebra species are closely related genetically (Kefena et al. 2012). Following their speciation during the early Pleistocene, there were episodes of species sympatry, as evidence by both zebra species appearing in the same Kenyan fossil assemblages an estimated 900–700 kyr ago (Potts et al. 1988) and again in Kenya 14–10 kyr ago (Marean 1992). Nevertheless, following the increase in aridity in East Africa approximately 600 000 years ago (deMenocal 2004), ancestral Grevy's zebras living in drier habitats would have arguably experienced prolonged relaxed selection from lions based on estimates of historical lion distribution (Bauer & Van Der Merwe 2004; Yamaguchi et al. 2004). The fact that mixed-species groups emerge even though Plains and Grevy's zebras occupy markedly different ecosystems outside of the overlap zone suggests behavioral compatibility for, and potential adaptive value of, grazing in close proximity.

Grevy's zebras may use alarm signals less often, due to being a less (consistently) social species. Additional support for this possibility is suggested, albeit indirectly, by Kimura's (2000) study of species differences

in certain social behaviors between Grevy's and Grant's (a subspecies of Plains) zebras. Kimura found that Grant's zebras performed mutual grooming and chin-resting behaviors significantly more frequently than did Grevy's zebras. Notably, when these results were discussed in greater detail, the subset of Grevy's that was allopatric to Grant's (and lived a territorial, socially scattered lifestyle) never performed either behavior, and the subset of Grevy's that was sympatric with Grant's (and lived in social groups that changed size with the seasons) occasionally performed chin-resting.

Although in our study, zebras in single-species groups displayed different (steeper) regression slopes for the relationships between FID and alert distance than the consistent  $\sim 0.44$  observed by Gulbransen et al. (2006), they were also remarkably consistent (see Fig. 5). Neither species nor group type differed appreciably from the overall slope of 0.77 (Table 1). This pattern implies that once alert distance is known, FID may be to some extent deterministic, and thus, efforts to learn how to predict alert distance will be a most useful strategy to researchers and wildlife managers who wish to predict FID. Alert distance may also be more important than FID for applied purposes, because it indicates when an animal is first (to our knowledge) affected by and possibly distressed by human presence, before it performs the more extreme and costly reaction of fleeing. As such, buffer zones for game drives and walking safaris would be thus more wildlife-friendly and more informative regarding species-specific needs if they took into account alert distances as well as actual flight behavior, a perspective echoing the viewpoint of Fernández-Juricic et al. (2001).

It is worth pointing out a possible methodological reason for our results regarding buffer distance differing from those of Fernández-Juricic and colleagues (Fernández-Juricic et al. 2001, 2002). Their research with bird species in urban parks was under much more controlled field conditions where start distance could be effectively kept constant by the researchers, and it is also likely that birds in urban parks are relatively more habituated than zebras in Africa at sites with low human density.

### Intermediate Behavior in Mixed-Species Groups

By deliberately choosing a specific target individual for each trial, we can compare the alert and flight behaviors of Plains and Grevy's zebras in mixed-species groups. Based on our findings, we posit that the intermediate levels of these behaviors exhibited by

each species within mixed-species groups, compared with their marked differences across single-species groups, characterize a moderating influence of nearby heterospecifics on threat appraisal.

Keeping in mind the small sample sizes of mixed-species groups, we must interpret the observed pattern of results with caution, considering that other undetermined factors correlated with threat assessment might influence the apparent structure of the two species' responses to each other. However, in light of the historical episodes of Plains and Grevy's zebra sympatry following species divergence and the likelihood of herd intermixing, it is reasonable to consider that the two species evolved proclivities to attend closely to each other's signals and behaviors. This argument is consistent with evidence of Plains–Grevy's hybridization (Cordingley et al. 2009), whereas other interspecific groupings for migratory purposes, such as Plains zebras and Wildebeests aggregations, might be alerted to predatory threats only by more salient auditory signals and flight behavior (Keast 1965; Sinclair 1985). Our finding that Plains zebras foraging with less attentive Grevy's zebras had significantly shorter alert distances than when foraging in Plains-only groups suggests contagious inattentiveness. Conversely, Grevy's zebras mingling with more attentive Plains zebras showed a less pronounced increase in alert distance, but this increase was not reliably different from the Grevy's single-species group, a property that might reflect less intent monitoring of alert signals from Plains zebras.

### Zebra responses to humans as potential predators

It appears that Plains zebras in areas of low human density are concerned about a human approaching them (Brubaker & Coss 2015). Both species kept an average flight distance  $>60$  m, which according to Schaller (1972) is the distance at which lions try to approach if they are hunting (for habitat variation, see Valeix et al. 2009). Lions are serious threats, although wild dogs can dispatch foals or weakened individuals if they hunt as a team, which is their typical hunting strategy. As noted above, Rubenstein (2010) found that, at least in some areas, Grevy's zebras are disproportionately preyed upon by lions; yet it is possible that Plains zebras classify humans as a serious threat in a 'lion-like' category, and flee early on, whereas the larger Grevy's zebras construe humans as a potential threat in a 'wild dog-like' category and respond by standing their ground, fanning out in a semicircle, ready to kick, and defend themselves and their young. Our observation that only

Grevy's zebras performed group fanning behavior is consistent with this notion, as is the anthropological factor that humans, if employing teamwork as a hunting technique, are in some ways more ecologically similar to African wild dogs (Marlowe 2005).

In conclusion, Plains and Grevy's zebras appear to uphold their anecdotal reputations for being more and less flighty, respectively, in response to human presence. Plains zebras also had a tendency to show overt alertness sooner than Grevy's did, even when controlling for their typically longer start distances. We have posited that, compared with Plains zebras in same-species groups, the reduction in alert and flight behavior recorded in Plains zebras grazing with Grevy's zebras to an approaching human likely reflects their more intensive monitoring of group behavior, especially the reduced defensive behavior of neighboring individuals. The contrasting increase in flight distance by the less social Grevy's zebras in mixed-species groups is only suggestive, but does encourage further study of the social dynamics in which one species monitors another in mixed-species groups.

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