Supplementary Material for:

Kin recognition for incest avoidance in Damaraland mole-rats, *Fukomys damarensis*

Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2024.1138

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S1. Methods: Behavioural observations

Table S1A. Mole-rat ethogram.

Behaviour	Description
Pump	Rapid up-and-down movement of the rear body.
Submission	Production of a high-pitched squeak co-occurring with a tremble of the body and/or jump backwards.
Self-groom	All behaviours with a hygienic or self-comforting function directed to the own body.
Groom	One individual grooming another.
Bite	One individual biting another.
Pull Tail	One individual biting the tail and pulling another. This differs from 'bite' as the bitten animal opposes to the motion of the biting animal.
Sparr	Locking incisors, pulling, pushing and chasing, with individuals facing one another (one reversing, one moving forwards).
Chase	One individual rapidly running after another.
Overt Aggression	A suite of rapid and behaviours intended to harm. Often associated with chasing from the aggressor, and submission from the receiver.
Sniff	Holding nose to an object, individual, or parts of the tunnel system, or sniffing the air with the head oriented upwards.
Pass	One individual passing another in the tunnel.
Sex Foreplay	Drumming, along with the rapid succession of bites, sparring, sniffs and passes. The rapid suite of behaviours is recorded as sex foreplay when associated with drumming, otherwise as individual behaviours.
Copulation	One individual mounting another and attempting intromission with pelvic thrusts.

Table S1B. Selected behaviours recorded during focal sessions.

Behaviour	Sampling technique
Self-groom	State
Sex Foreplay	State
Sparr	State
Bite	Event
Copulation	Event
Overt Aggression	Event
Sniff	Event

Table S1C. Selected behaviours recorded during scan sessions.

Behaviour	Sampling technique	Comment
Social Interaction	Instantaneous	Any of: groom, bite, pull tail, sparr, chase, overt aggression, sniff, pass, sex foreplay, copulation.
Pump	Instantaneous	
Self-groom	Instantaneous	
Groom	Continuous	
Bite	Continuous	
Pull Tail	Continuous	
Sparr	Continuous	

Chase	Continuous	
Overt Aggression	Continuous	Overt aggression occurring during instantaneous scans was recorded continuously.
Sniff (social)	Continuous	Touching another animal with the nose.
Pass	Continuous	
Sex Foreplay	Continuous	Sex Foreplay occurring during instantaneous scans was recorded continuously.
Copulation	Continuous	Copulation occurring during instantaneous scans was recorded continuously.
Submiss	Continuous	
Pump	Continuous	

Table S1D. Summary of observation session duration (mins).

Observation Session	**Mean ± SD**	**Range**
Day 0 Focal	85.34 ± 25.4	60.02-120.11
Day 1 Focal	59.03 ± 6.83	22.33-61.5
Scan	704.27 ± 12.15	604-728

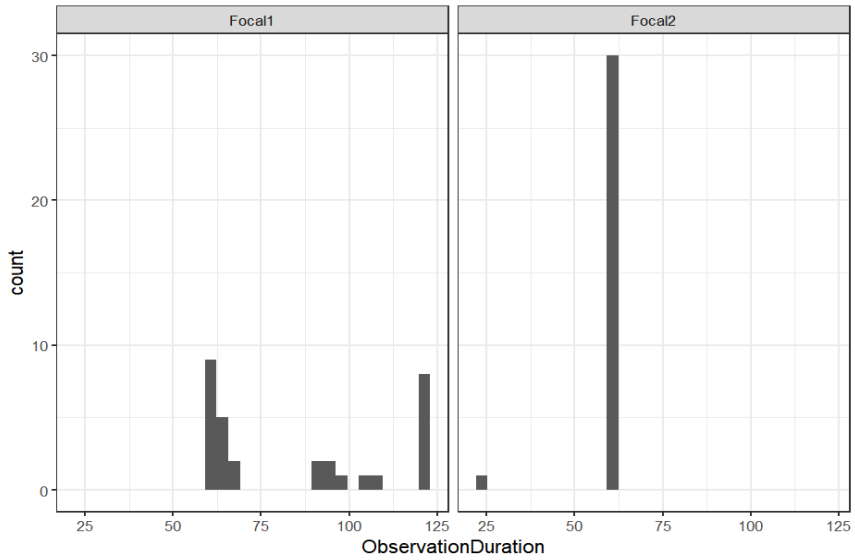


Figure S1E. Observation durations (mins) of focals on Day 0 (Focal1) and Day 1 (Focal2).

S2. Methods: Reproductive physiology

Sample preparation for hormone quantification

50 or 100 µl of urine were added to 400 µl of a solution containing 390 µl of sodium phosphate buffer (0.1M, pH7) and 10 µl of methanol (MeOH) spiked with isotopically deuterated-labelled internal standards of progesterone-d9 (50ng/ml), estradiol-d4 (50ng/ml), cortisol-d4 (50ng/ml), testosterone-d3 (20ng/ml), androstenedione-d7 (50ng/ml) and dehydroepiandrosterone-d5 (500ng/ml) purchased at Toronto Research Chemicals. Isotopically labelled hormones have similar chemical properties than their non-labelled counterparts, which allows to correct for losses during sample preparation, matrix effects and variation in mass spectrometer's sensitivity over time (Stokvis et al., 2005). 2.5 µl of beta-glucuronidase from *Escherichia Coli* (Roche chemicals) was added to each sample extract and left to incubate 1h at 50 ºC to deconjugate the glucuronidated forms of steroids. Glucuronidated steroids are more soluble in water and represent the main excretion forms of steroids in urine (Schanzer, 1996). Immediately after incubation, a solid phase extraction (SPE) using Isolute C18(EC) columns (50 mg/1cc, Biotage, Sweden) was performed. Columns were first conditioned with 1 ml of methanol (MeOH) and equilibrated with 1 ml of MeOH 5%. Samples were then run on the columns followed by a wash with 1 ml of MeOH 5% and 1 ml of hexane. Steroids were recovered by eluting the columns with 1 ml of ethyl acetate in an Eppendorf tube which were placed in a centrifugal evaporator (Labconco) at 35 ºC until complete evaporation (approx. 45 minutes). Dried extracts were reconstituted in 100 µl of MeOH 50%, vortexed (20s), ultrasonicated (1min) vortexed again (20s), centrifuged 3min at 13'000rpm before being transferred in HPLC vials.

Ultra-High Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC-MS/MS)

For UHPLC-MS/MS analyses, samples were injected in an Acquity UPLC™ I-Class coupled to a Xevo TQ-XS triple quadrupole (Waters, Milford, MA, USA) with all aspects of the system optimized for steroid analyses (Binning et al., 2017; Vullioud et al., 2021). Calibration solutions containing 0.1, 1, 5, 20, 100, 250, 500 ng/ml of every hormone and their respective internal standards (at identical concentrations to sample extracts) were prepared in MeOH 50%. The system was controlled by Masslynx 4.2. Chromatographic peaks were integrated using the program Targetlynx™ and normalized to those of the internal standards. Peak integrations were visually controlled for each sample and each hormone. Calibration equations were

independently applied to each hormone by batch, where the best fitted model (linear or quadratic) and weighting factor (1/x or $1/x^2$) were separately selected for lower concentrations (<= 100 ng/ml) and higher concentrations (>100 ng/ml).

All concentrations with a signal/noise ratio >= 3 (limit of detection, LOD) were defined as detectable and raw concentrations were used, whereas concentrations with a signal/noise ratio <3 (LOD) were defined as non-detectable and assigned a value of 0. Although concentrations determined from signal/noise between 3 and 8 (limit of quantification *sensu stricto*, LOQ) are less accurate that concentrations >LOD, we decided using raw concentrations over a fixed assigned value, such as 0 or overall methods' LOD, to avoid unnecessary complications of statistical models. This is acceptable as data explorations revealed that intended comparisons were to be made between hormone levels that mostly fell > LOD and levels that mostly fell < LOD, the former being several orders of magnitude higher than the latter.

Coefficient of variation of hormone concentrations

The coefficient of variation of quantifiable hormone concentrations (CV = (standard deviation/mean) * 100) in control and non-control urine samples were calculated to determine the robustness and reproducibility of our quantification methods.

Prior to analyses, a male (MC) and a female (FC) control sample were constituted by separately pooling urine samples from many captive Damaraland mole-rats of each sex. MC and FC were separated into 1ml aliquots and stored at -20°C until analyses. MC and FC samples were prepared for analyses as any other urine samples and independent extracts were quantified once. One extract was placed ne MC and one FC were placed on each batch, at the exception of one batch that received two extracts (n= 7 batches, n=8 MC and MF extracts). CV of MC and FC were calculated from concentrations that fell above the LOQ, returning a CV of 4.56% for E2 and 4.15% for P4 (Table S2A). We also computed the CV from non-control samples which concentrations had been quantified more than once using independent urine extracts. The CV of samples were averaged over each hormone, returning a mean CV of 9.79% for E2 and 5.74% for P4 (Table S2B; Figure S2C).

Table S2A. Coefficient of variation (CV) of raw hormone concentrations calculated using concentrations in extracts from a female control (FC) and a male control (MC) sample.

Sample ID	Hormone	Count of extracts with quantifiable concentrations	Mean concentrations [ng/ml]	CV in %
FC	E2	8	40.47	4.56
FC	P4	4	0.15	4.15

Table S2B. Mean coefficient of variation of raw E2 and P4 concentrations calculated using non-control urine samples: For each sample, hormone concentrations were quantified in two or three extracts (replicates) that were independently prepared for hormone analyses using up to two distinct volume of urine and that were placed in up to two distinct batch of MS. In total, four different volumes of urine were used, and hormones were quantified across four distinct MS batches. Mean CV for a given hormone was calculated by averaging the CV of each individual sample for that hormone. Note that replicates of non-control samples were removed from dataset before statistical analyses.

Hormone	Distinct samples	Distinct extracts	Min. concentrations [ng/ml]	Max. concentrations [ng/ml]	Min. sample CV [%]	Max. sample CV [%]	Mean CV [%]
E2	15	37	1.18	562.788	0.13462	52.6907	9.78753
P4	9	21	0.068	1.118	0.43248	11.4666	5.74258

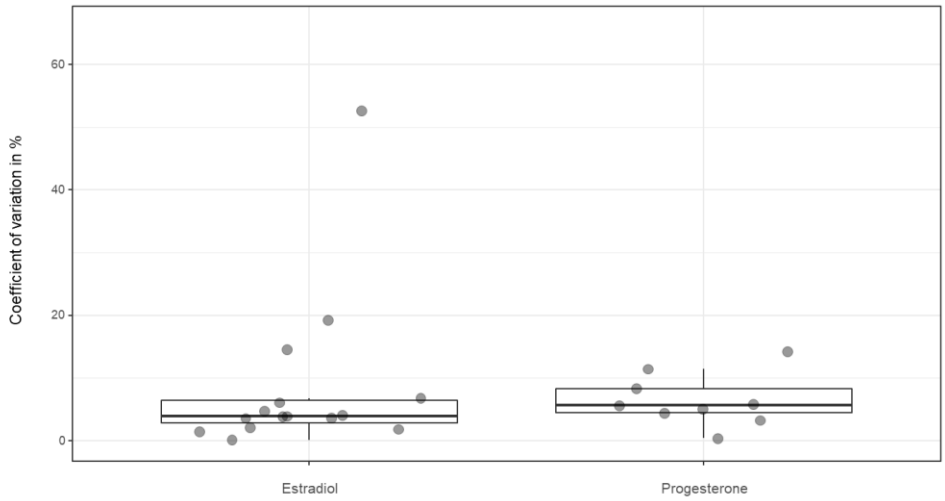


Figure S2C. Coefficient of variation of raw E2 and P4 concentrations determined from concentrations measured in independently prepared replicates of individual samples.

Raw hormone concentrations obtained from mass spectrometry were corrected to account for variation in the volume of urine extracted for hormone analyses. Hormone concentrations were then corrected for variation in urine dilution by the determination of urine specific gravity (SG) using a digital hand-held pen refractometer (Atago Ltd). Correction of hormone concentration with SG has been shown to be reliable and arguably more accurate than creatinine correction (Miller et al., 2004). SG was individually measured in 10ul sample extract. SG was determined at least in triplicates unless urine volume was insufficient. Hormone concentrations adjusted for variation in SG were obtained following Miller and colleagues (Miller et al., 2004):

$$[\text{Corrected Hormone}] = [\text{Raw Hormone}] \times (SG_{\text{Population}} - 1) / (SG_{\text{Target Sample}} - 1),$$

where $SG_{\text{Population}}$ is the average SG values of all samples used in our study and $SG_{\text{Target Sample}}$ is the SG value of the sample which hormone concentration is corrected.

Data exploration of hormone profiles

We expected reproductive activation to be highlighted by a transient pre-ovulatory surge in E2 followed by a longer-lasting elevation in both E2 and P4 indicative of a luteal phase or early pregnancy. Between individual variation in hormone levels and in the time course of hormonal changes complicated the visual detection of such patterns at the treatment level requiring the separate inspection of individual hormone profiles (see Figure S2A).

Visual inspection of individual E2 and P4 profiles showed that hormonal profiles of females paired with a familiar or unfamiliar males dramatically differed. Post-pairing E2 and P4 levels of females paired with a familiar male mostly (69.19% of E2 and 82.15% of P4 levels) fell below the LOQ. In all but one of these females, E2 and P4 levels remained consistently low relative to levels of females paired with an unfamiliar male. Overall, only five females paired with a familiar male had more than one of their E2 levels above the LOQ and three had more than one of their P4 levels above the LOQ. In contrast to females paired with a familiar male, E2 and P4 started rising within a few days or weeks after pairing in all but one female paired with an unfamiliar male. The initial rise in E2 and P4 coincided in time or occurred within a few days of each other. Following this initial rise, levels of both hormones generally kept increasing, or stayed elevated, for several weeks, never falling below their LOQ. The hormonal profiles of one female paired with a familiar male resembled the ones of females paired with an unfamiliar male (Figure 2SA – Waxbill), suggesting reproductive activation, though no life-history information suggested it ever got pregnant and it never produced a litter.

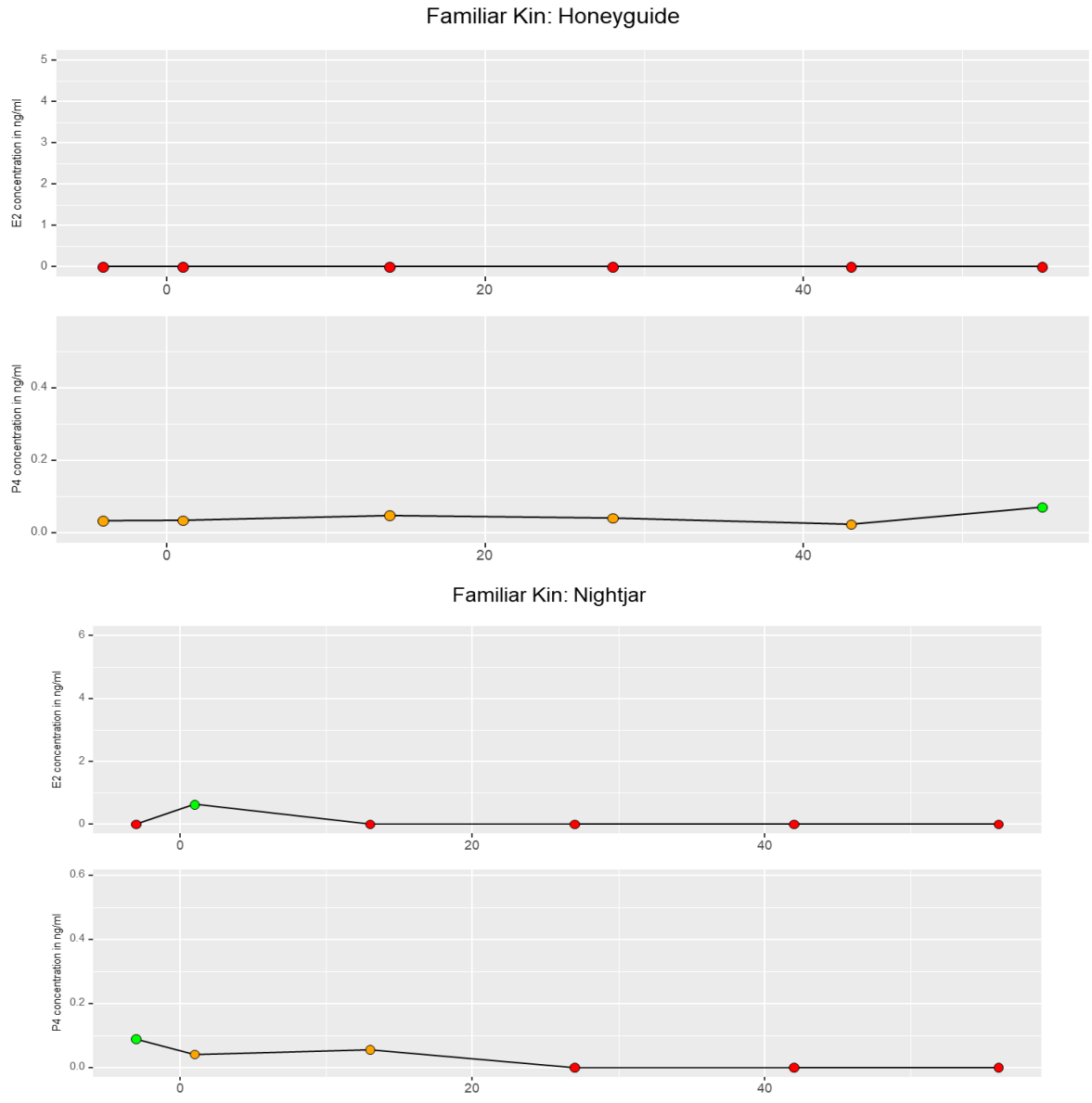
Individual hormone profiles did not seem to reveal, at least reliably, pre-ovulatory surges in E2 that could have been used to consistently time first ovulation across females. Only seven of the fifteen females which activated their reproductive axis displayed a transient surge in E2 prior to a sustained elevation in E2 and P4 suggestive of a luteal phase or pregnancy. In addition, for all but one of these females, P4 levels were already increased or quantifiable at the time of the transient surge in E2. This pattern contrasts with the expectation that increases in P4 should follow, not coincide or precede, pre-ovulatory surge in E2. We believe that the coverage of our urine sampling scheme (every 3 days post-pairing) was insufficient to capture pre-ovulatory surges in E2, causing their underlying hormonal signatures to blend with the ones of early pregnancy or luteal phases.

Determination of reproductive axis activation

The impossibility to consistently and reliably time first ovulation using pre-ovulatory surges in E2 forced us to determine threshold based E2 and P4 criteria to define and time reproductive activation. Due to the subjectivity of the process and the variability it may cause (see Figures S4F and G) , we developed four single hormone criteria (separately applied to both E2 and P4) and two combined hormone criteria (total = 10 criteria) upon which reproductive activation could be defined. These criteria were developed based on data exploration showing discrete differences in the hormonal profiles of females that produced and did not produced pups, and on the a priori prediction that reproductive activation should be associated with increases in E2 and P4. We developed these defining criteria based on hormonal profiles of females that produced pups. The timing of reproduction activation was defined as the earliest timepoint at which:

1. E2 levels surpass the highest post-pairing levels of females that did not produce pups; threshold > 8.996ng/ml.
2. P4 levels surpass the highest post-pairing levels of females that did not produce pups; threshold > 0.101ng/ml.
3. Criteria 1 fulfilled in two successive samples.
4. Criteria 2 fulfilled in two successive samples.
5. As criteria 1, but with thresholds increased to 120% of highest levels measured in females that did not produce pups; threshold > 10.80 ng/ml.
6. As criteria 2, but with thresholds increased to 120% of highest levels measured in females that did not produce pups; threshold > 0.12 ng/ml.
7. Criteria 5 fulfilled in two successive samples.
8. Criteria 6 was fulfilled in two successive samples.
9. Both E2 and P4 levels fell above their LOQ.
10. Criteria 9 was fulfilled in two successive samples.

These criteria likely captured E2 and P4 profiles associated with ovulation, conception, implantation or early gestation. Indeed, for females which reproductive activation was associated with a successful gestation, reproductive activation across the ten criteria was timed between 93 days and 79 days prior to first parturition (see Figure S4G), shortly after the time window of expected successful mating (100, 95, and 94 days prior to parturition, unpublished data). One female paired with an unfamiliar non-kin male (Hammercobb), which eventually produced a litter, did not activate its reproductive axis by 187 days post-pairing and was assigned the day of its next collected sample (190 days post-pairing) as an estimate of reproductive activation.



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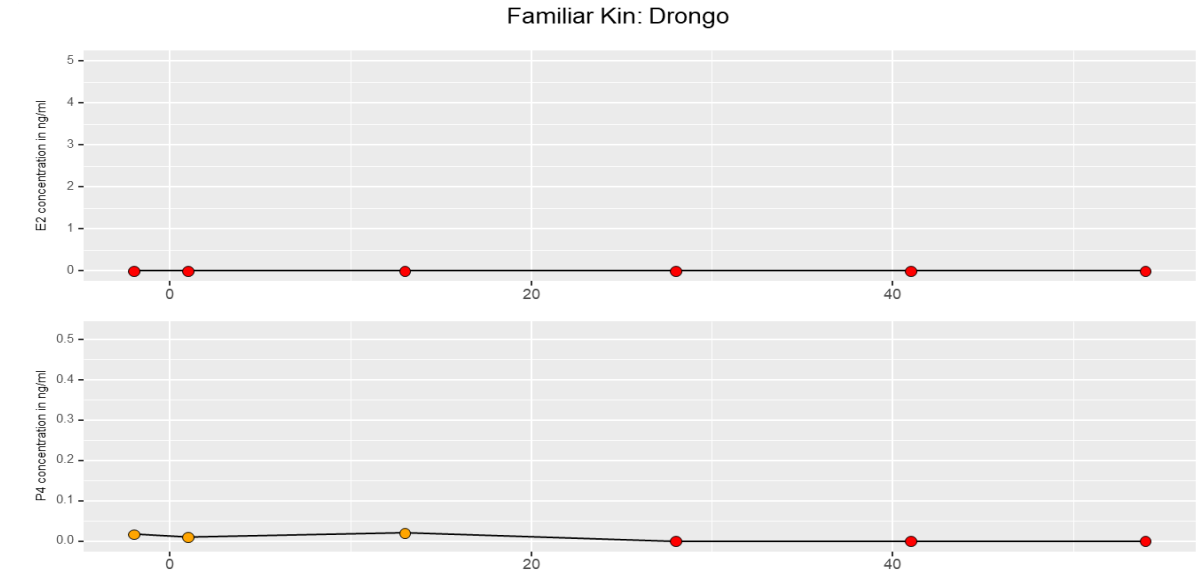
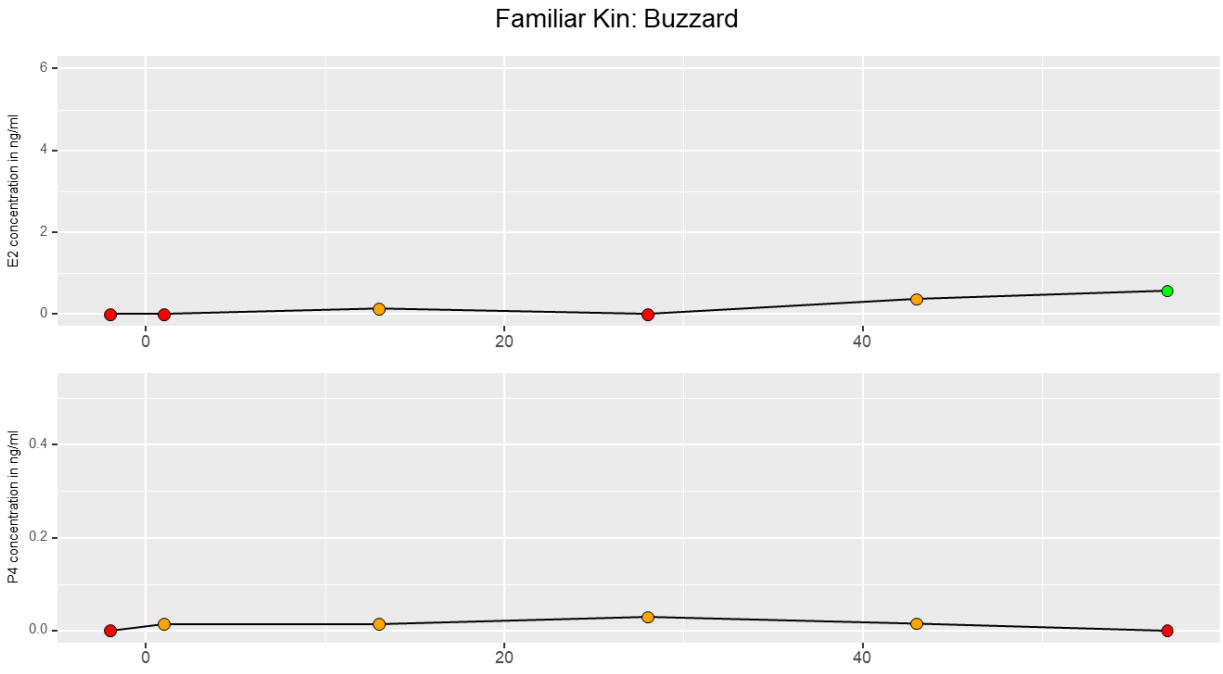
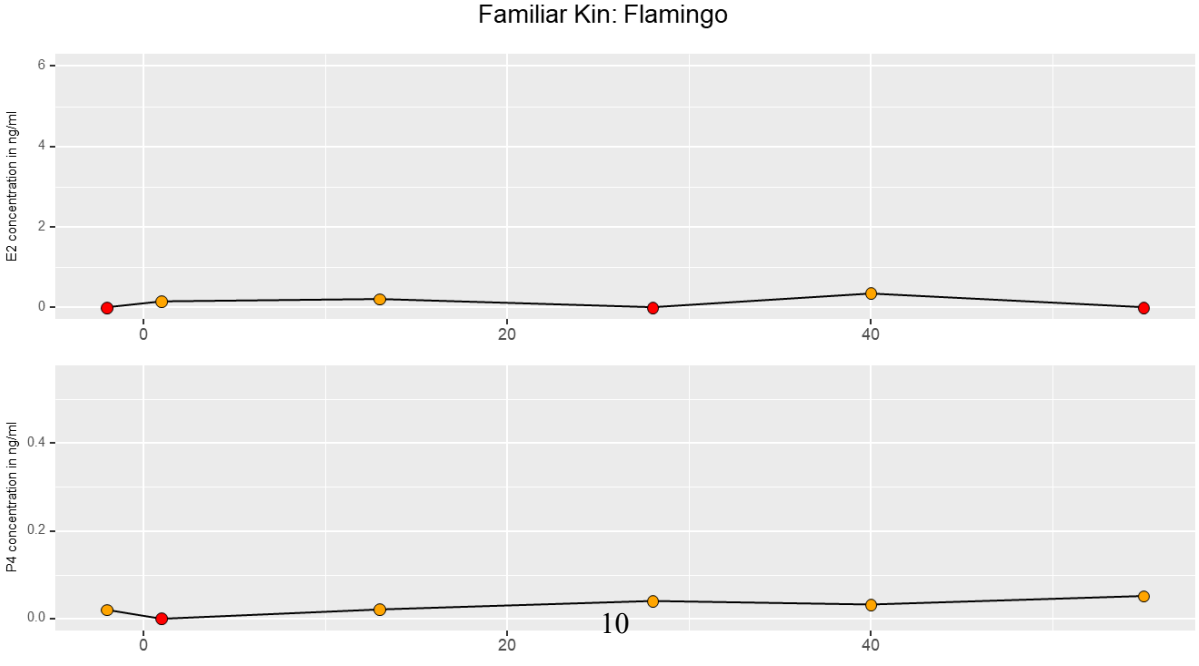
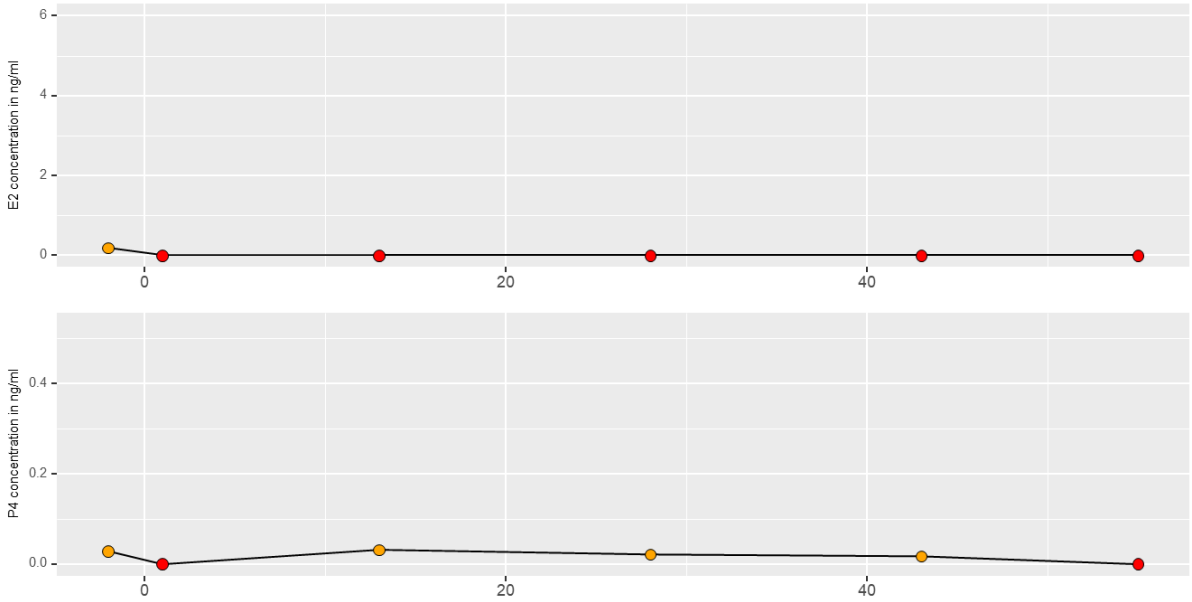


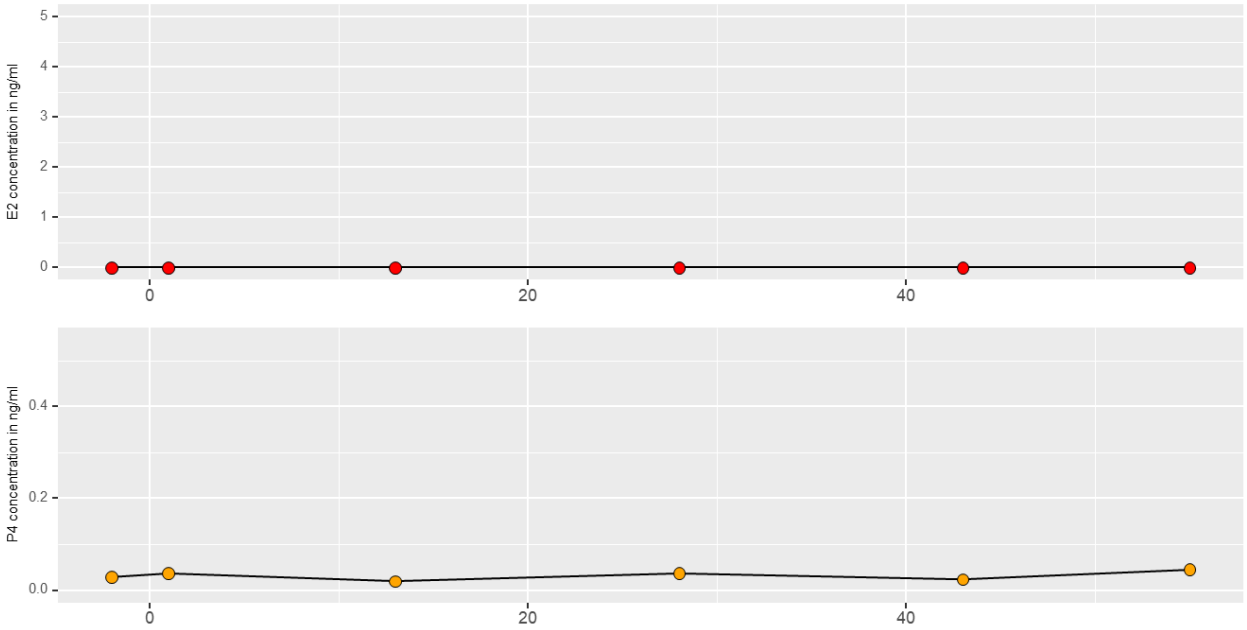




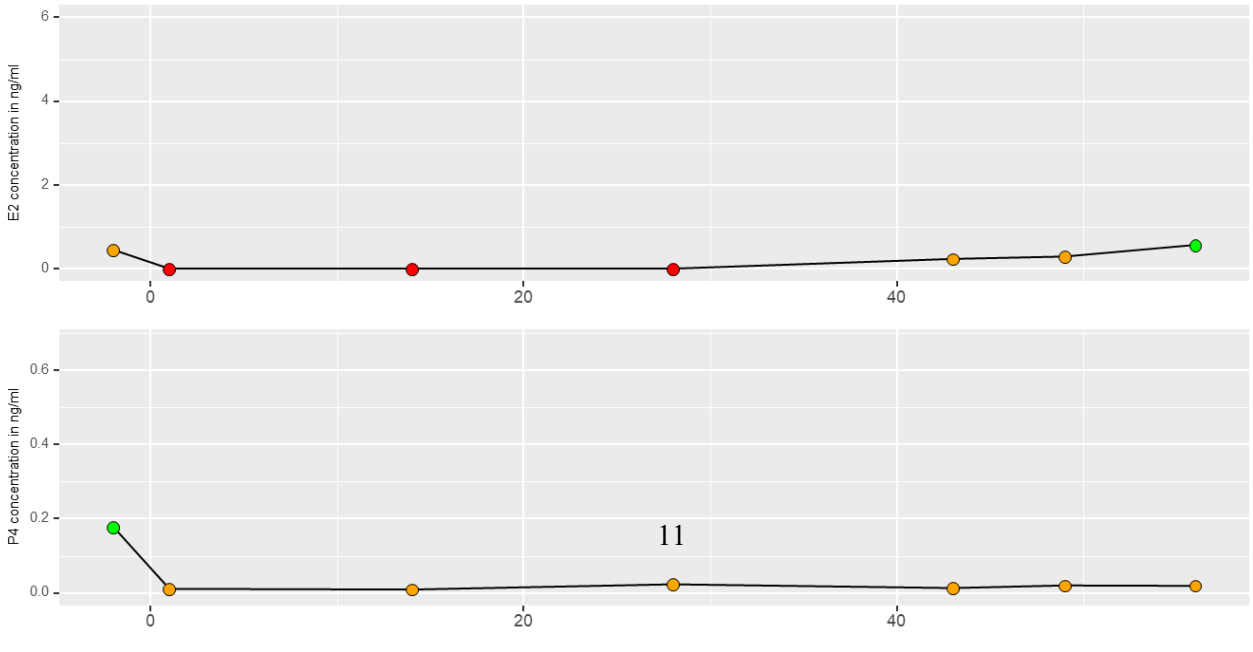
Familiar Kin: Ostrich

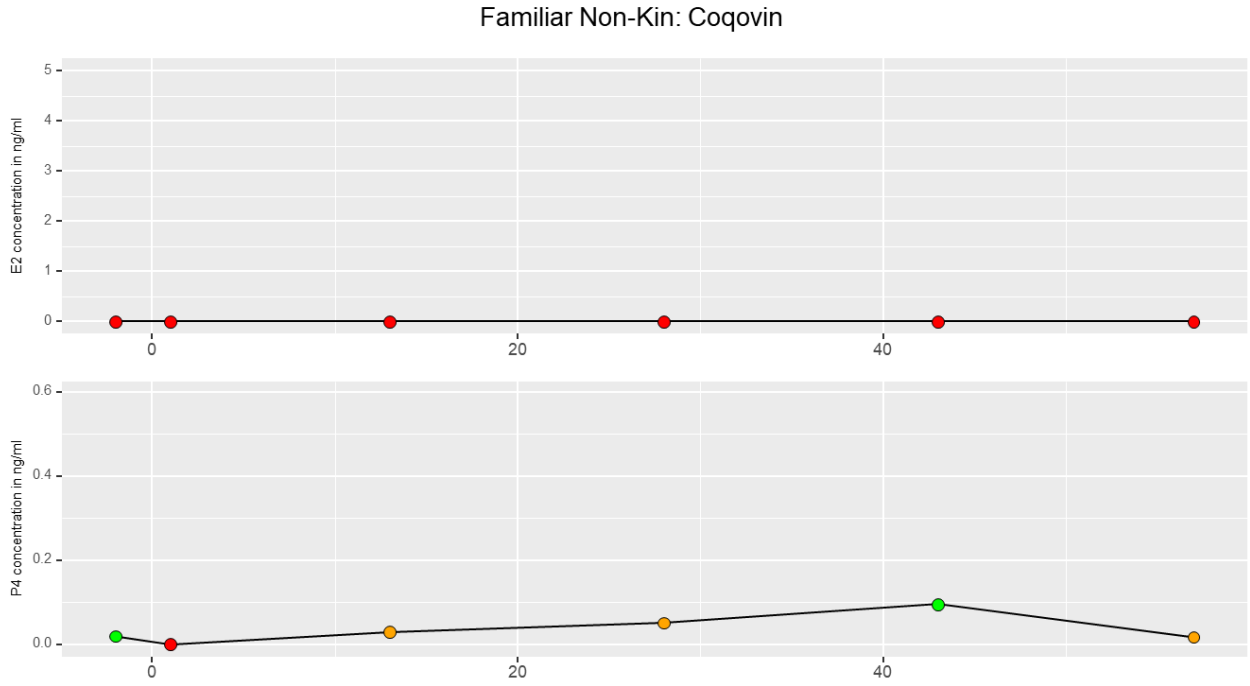


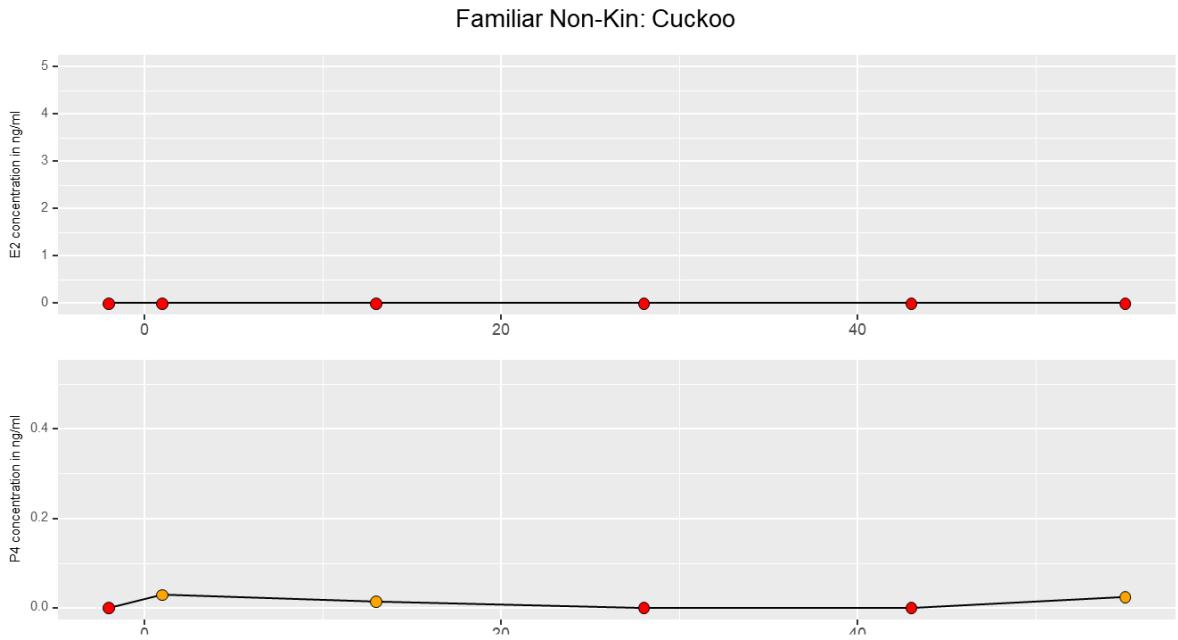
Familiar Kin: Pytilia

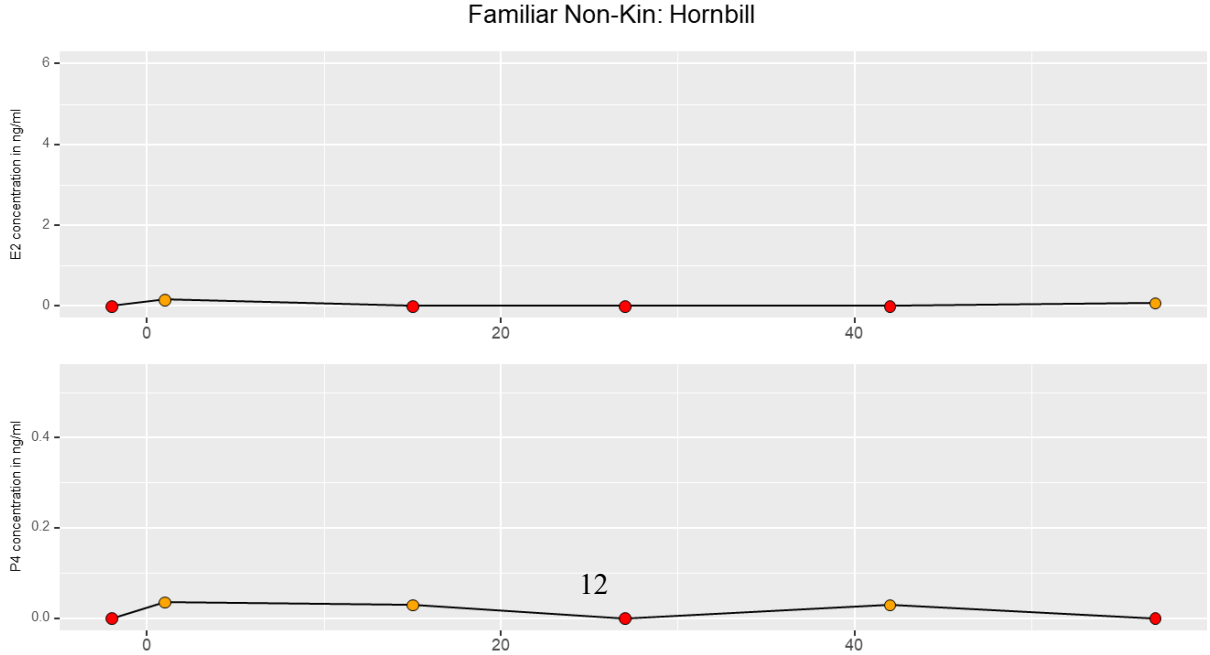


Familiar Kin: Brubru




Familiar Non-Kin: Coqovin
E2 concentration in ng/ml
P4 concentration in ng/ml


Familiar Non-Kin: Cuckoo
E2 concentration in ng/ml
P4 concentration in ng/ml


Familiar Non-Kin: Hornbill
E2 concentration in ng/ml
P4 concentration in ng/ml

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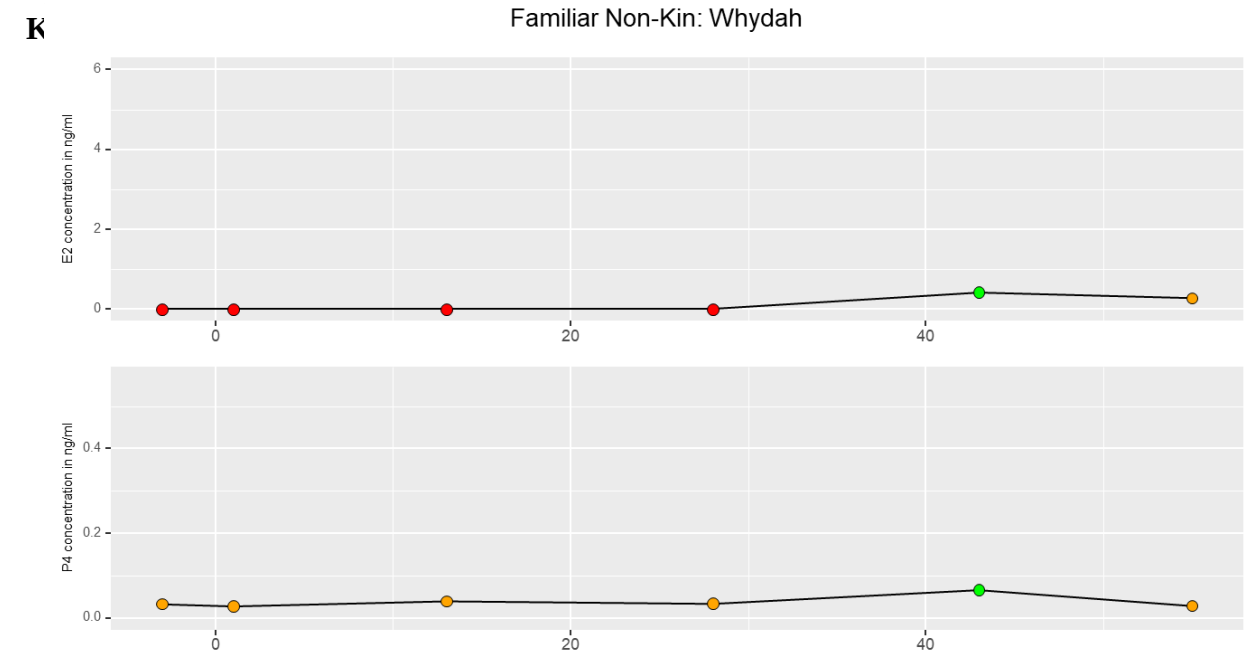
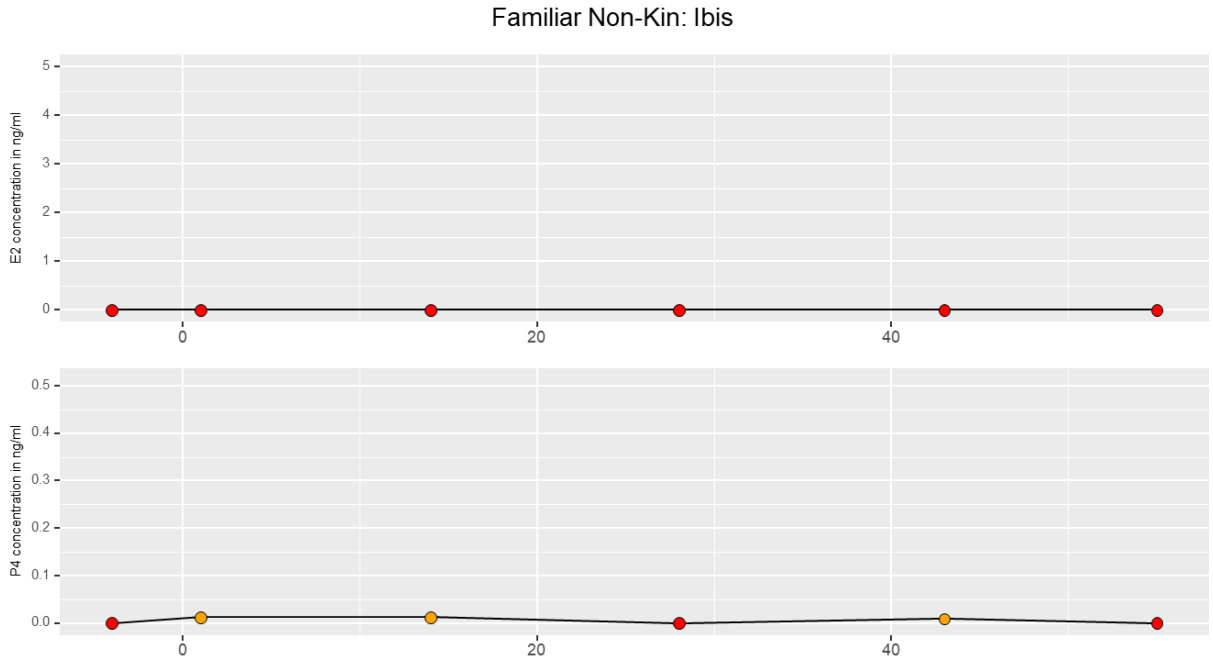
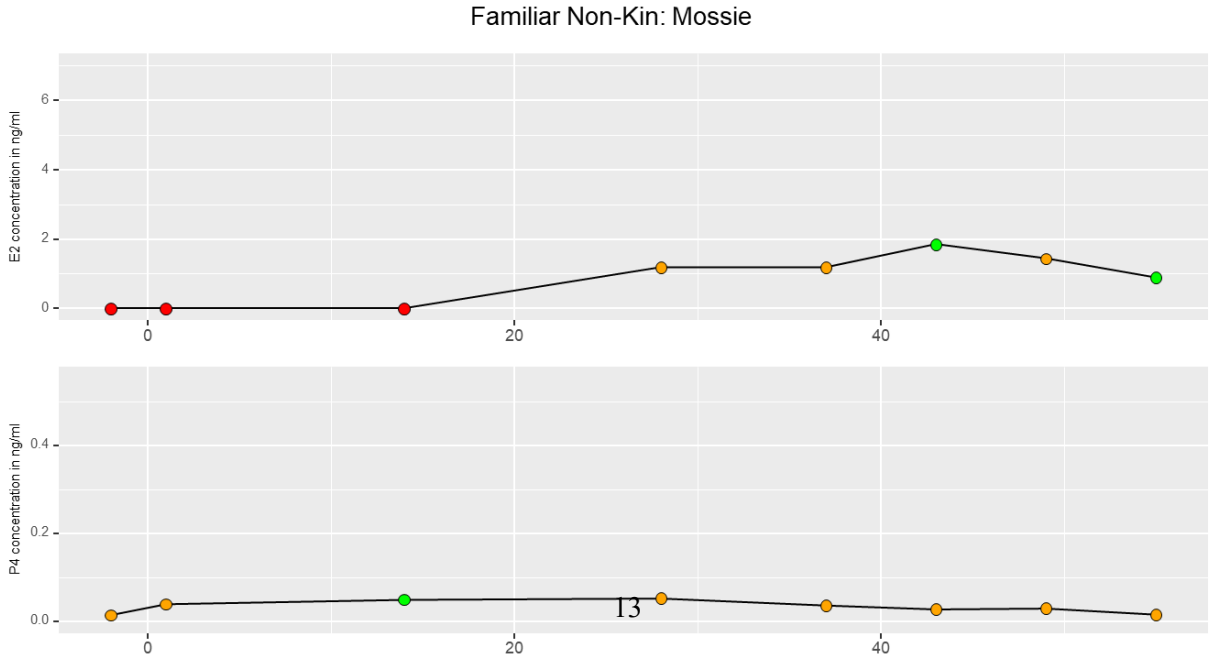
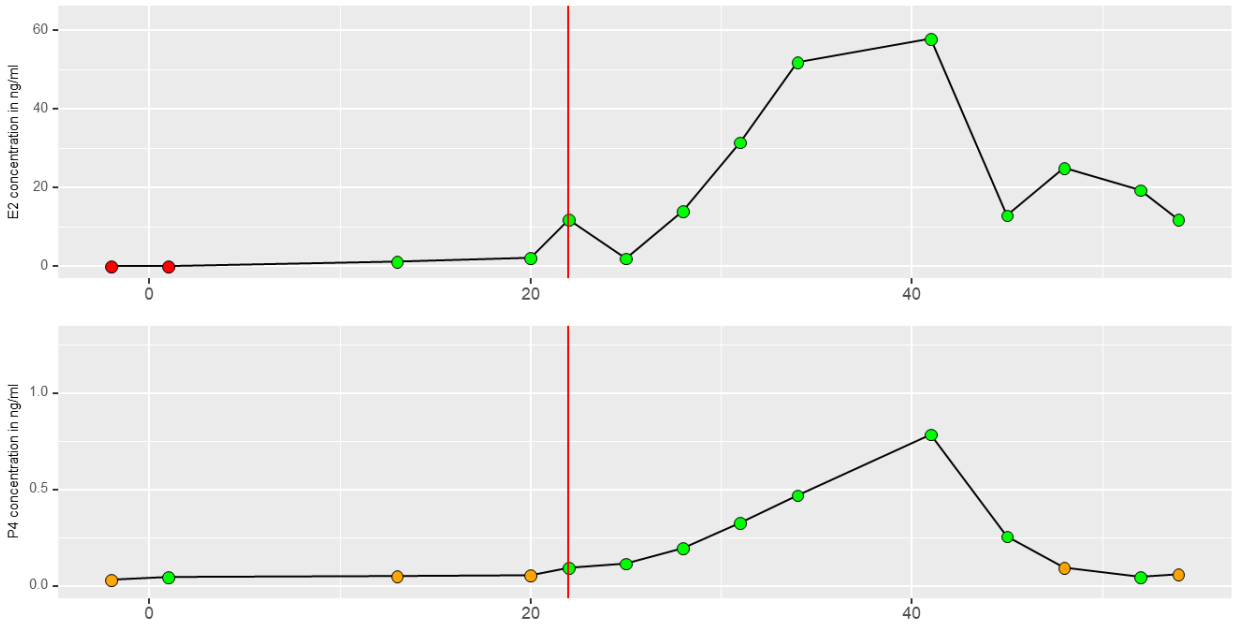

Familiar Non-Kin: Whydah
E2 concentration in ng/ml
P4 concentration in ng/ml

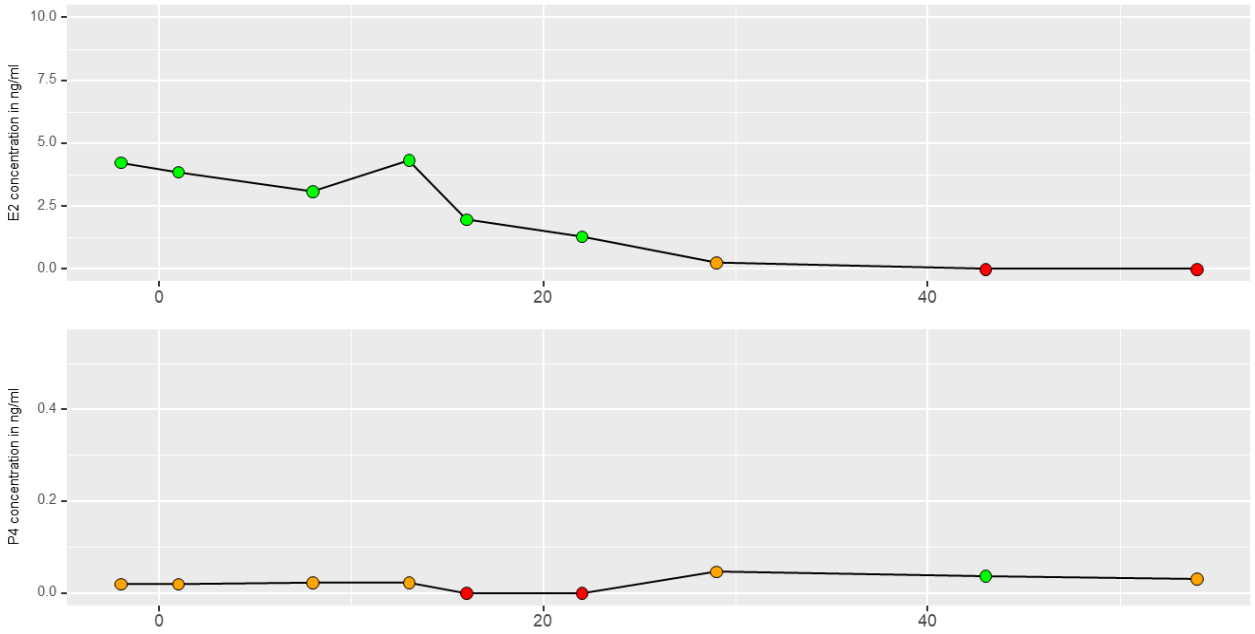

Familiar Non-Kin: Ibis
E2 concentration in ng/ml
P4 concentration in ng/ml


Familiar Non-Kin: Mossie
E2 concentration in ng/ml
P4 concentration in ng/ml

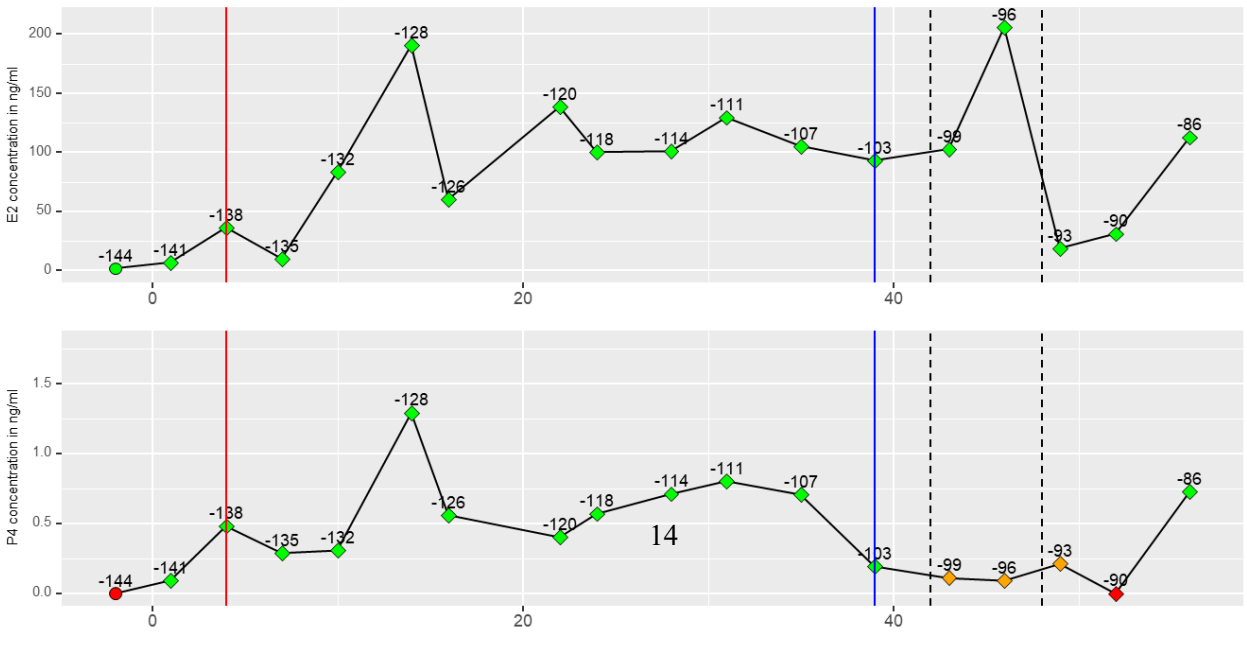
Familiar Non-Kin: Waxbill



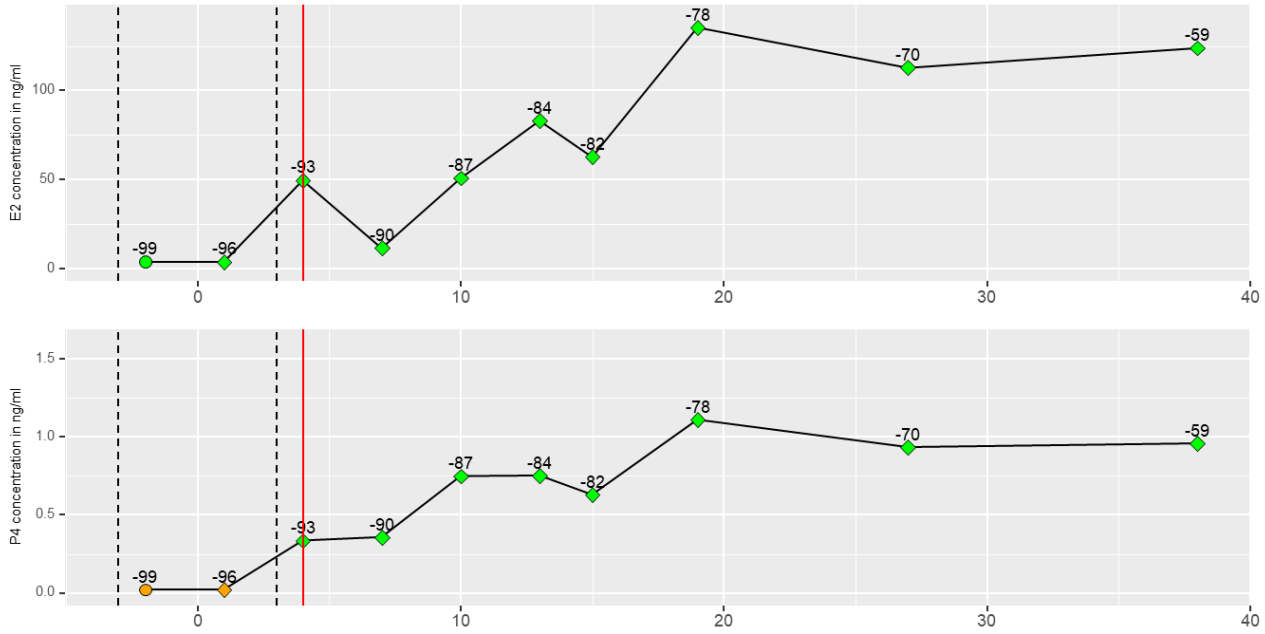
Familiar Non-Kin: Shrike



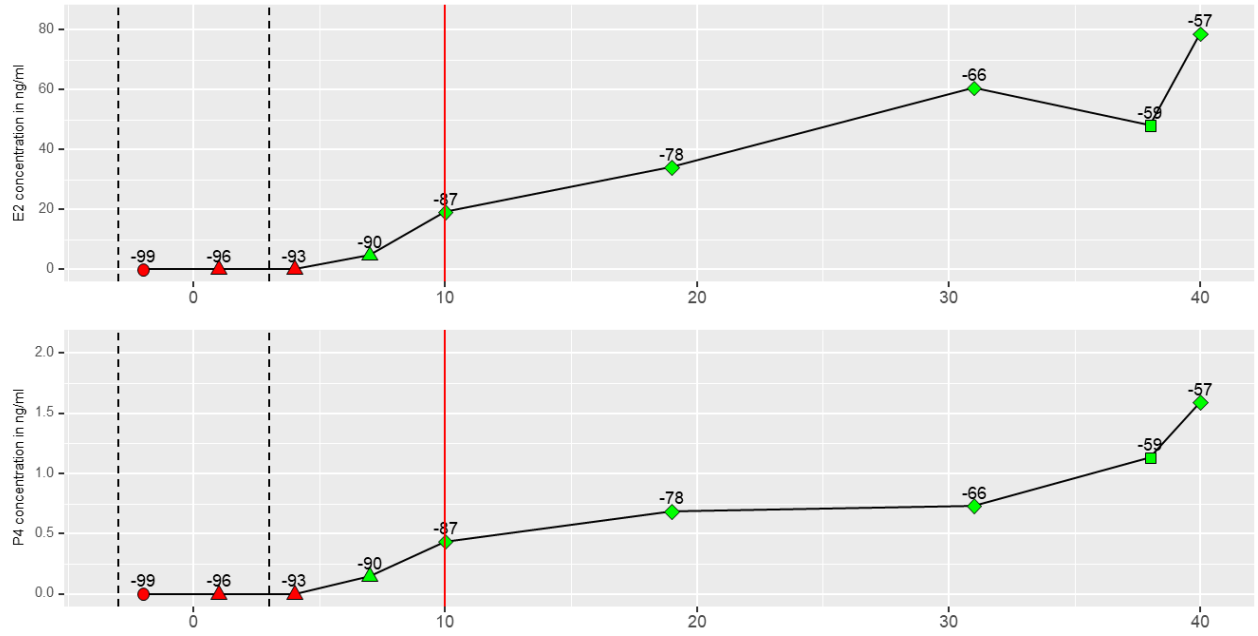
Unfamiliar Kin: Pigeon



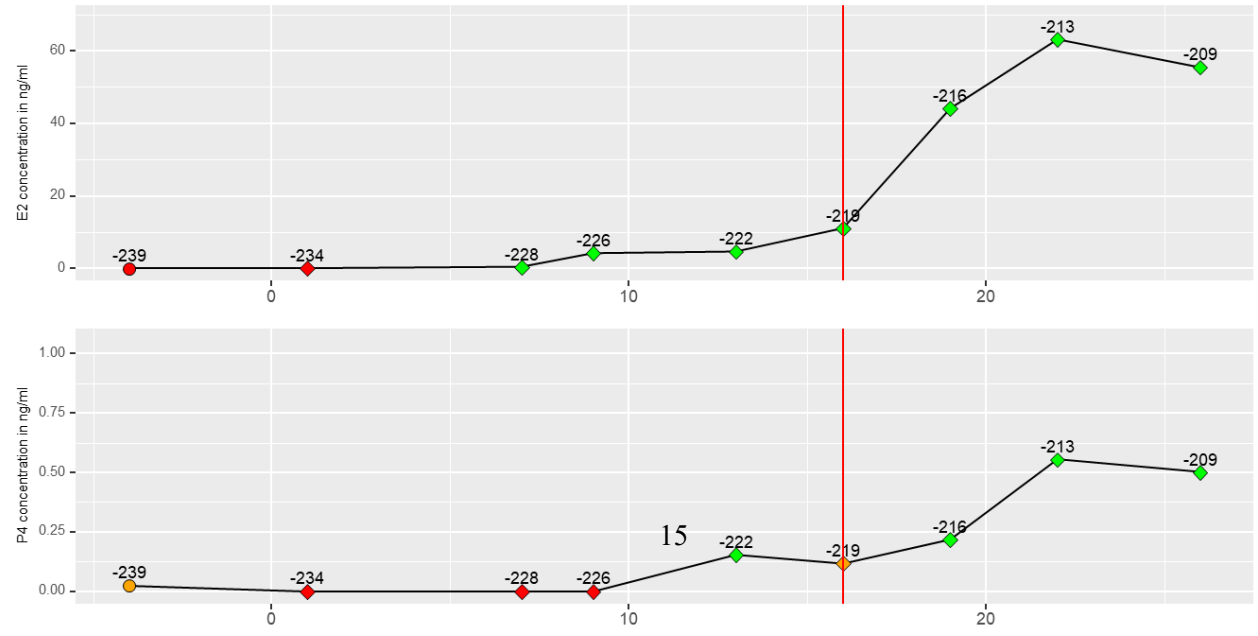
Unfamiliar Kin: Sneeubal



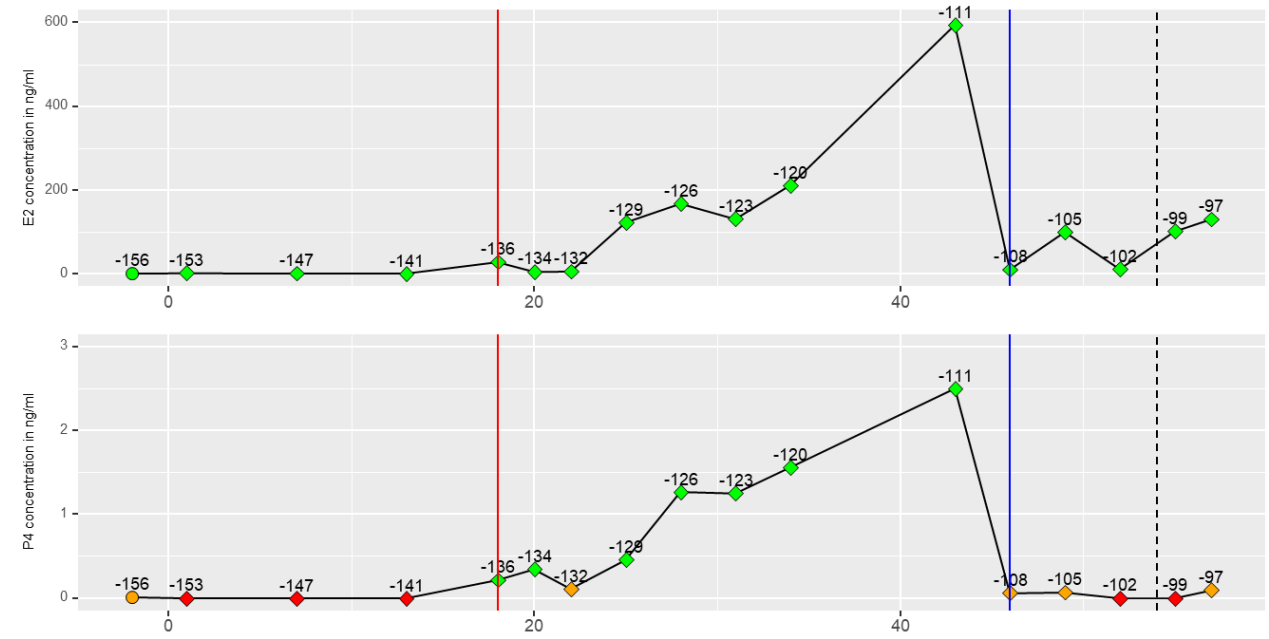
Unfamiliar Kin: Tit-Babbler



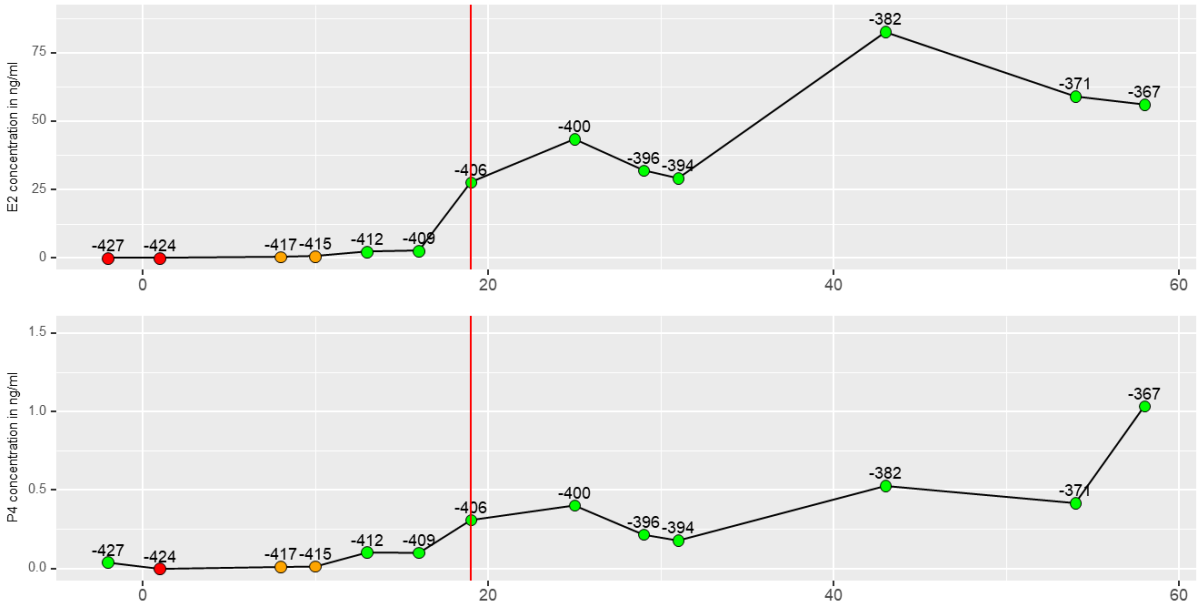
Unfamiliar Kin: Bishop



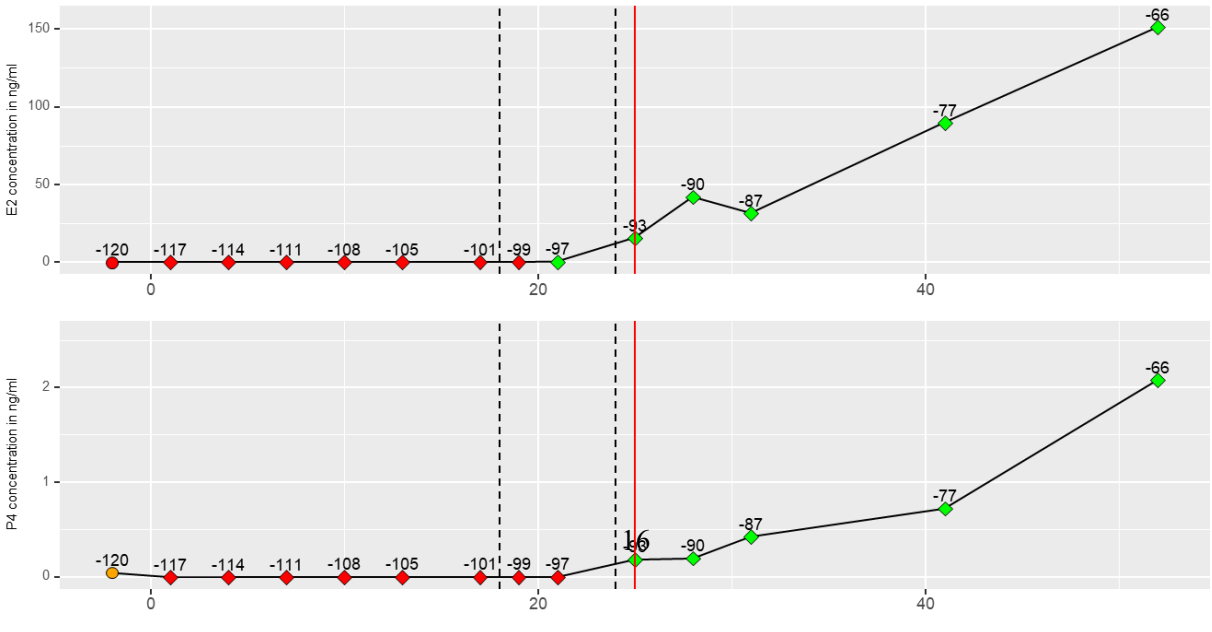
Unfamiliar Kin: Bateleur



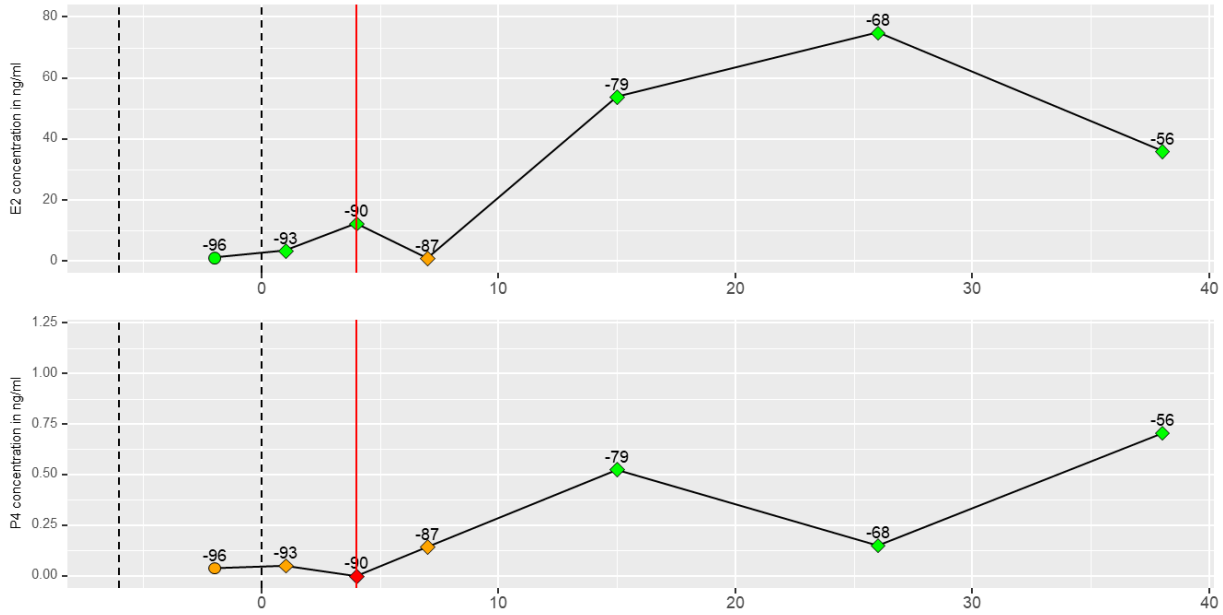
Unfamiliar Kin: Hoopoe



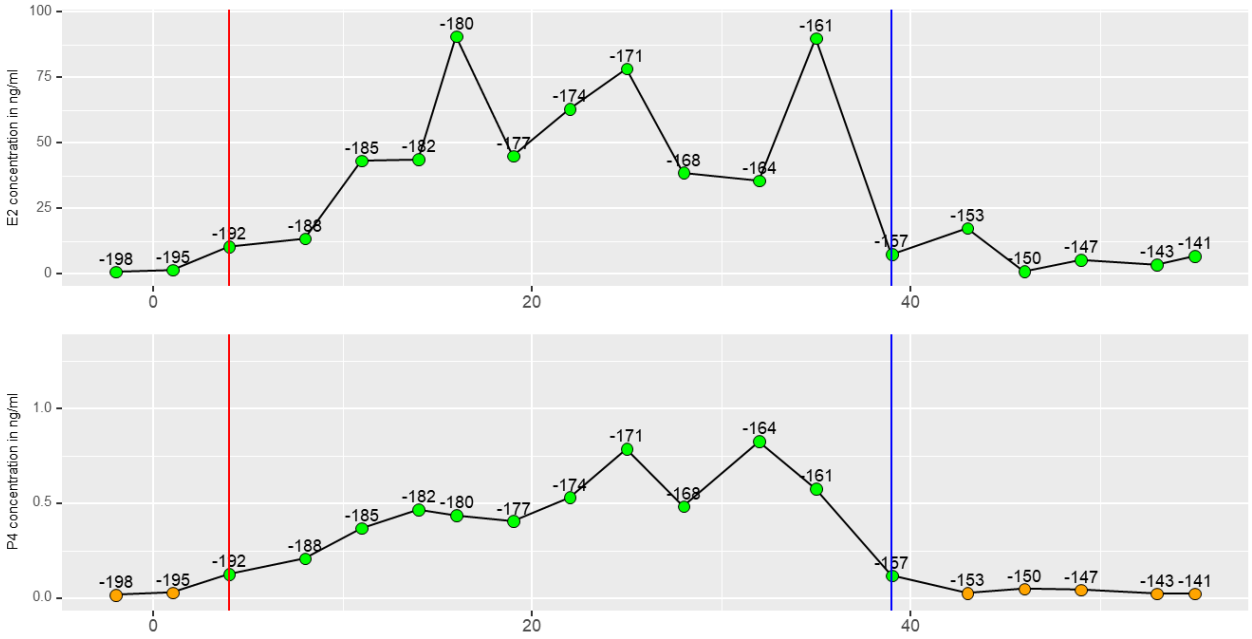
Unfamiliar Kin: Tinktinkie



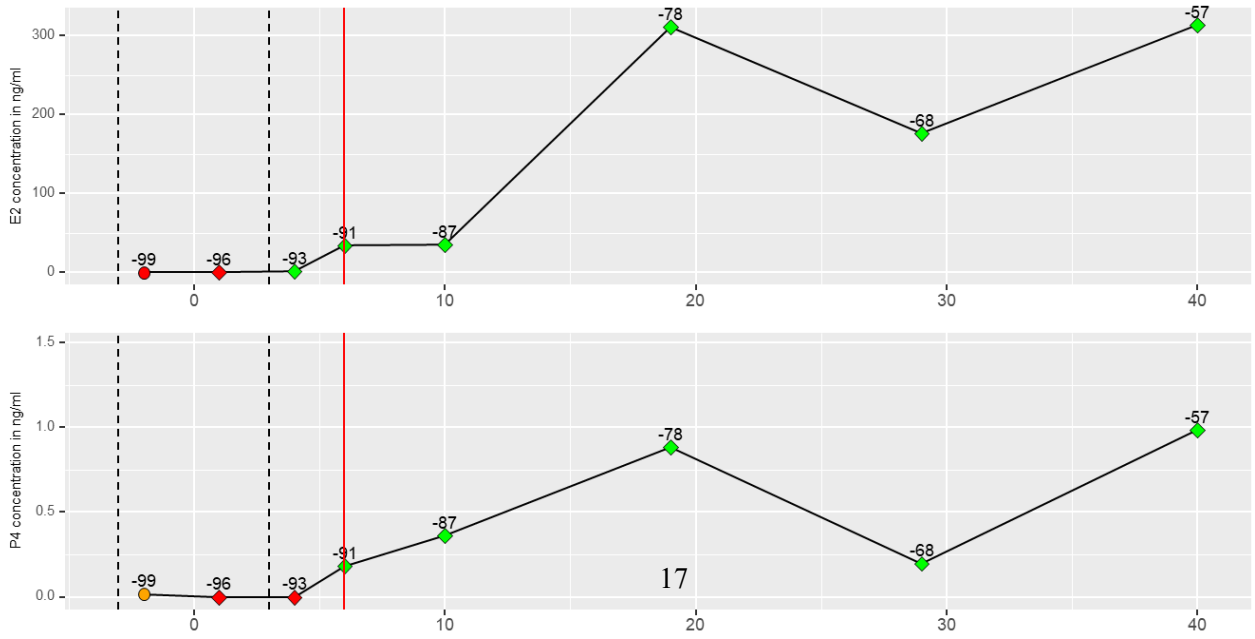
Unfamiliar Non-Kin: Kapantsi



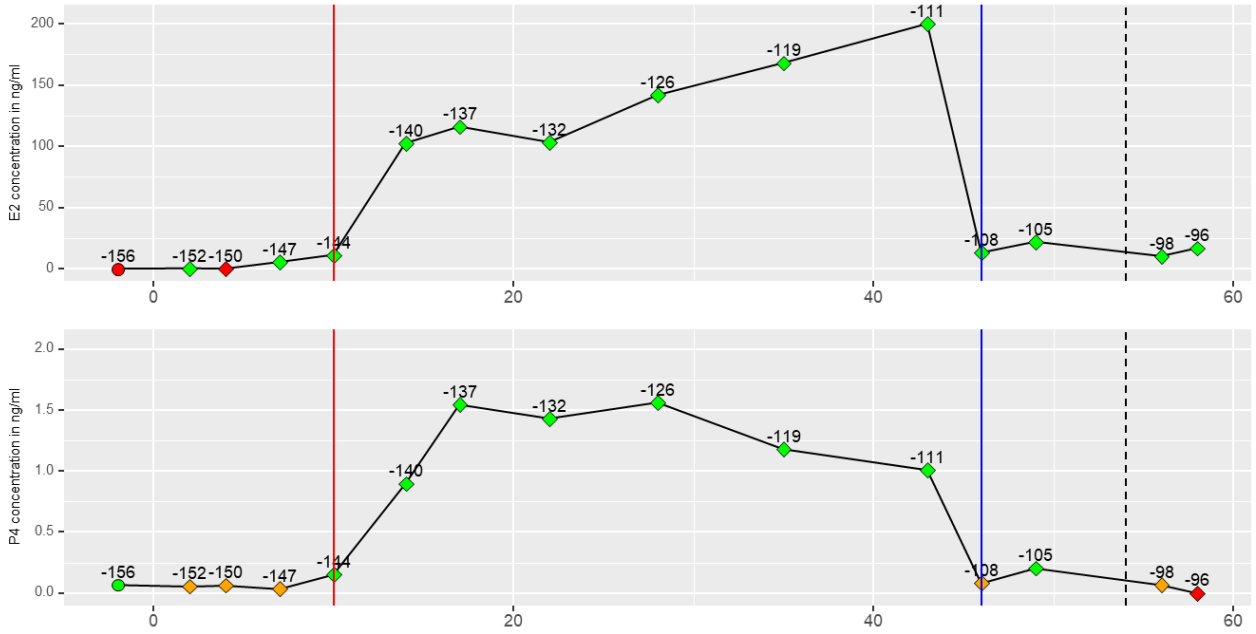
Unfamiliar Non-Kin: Bokmakerie



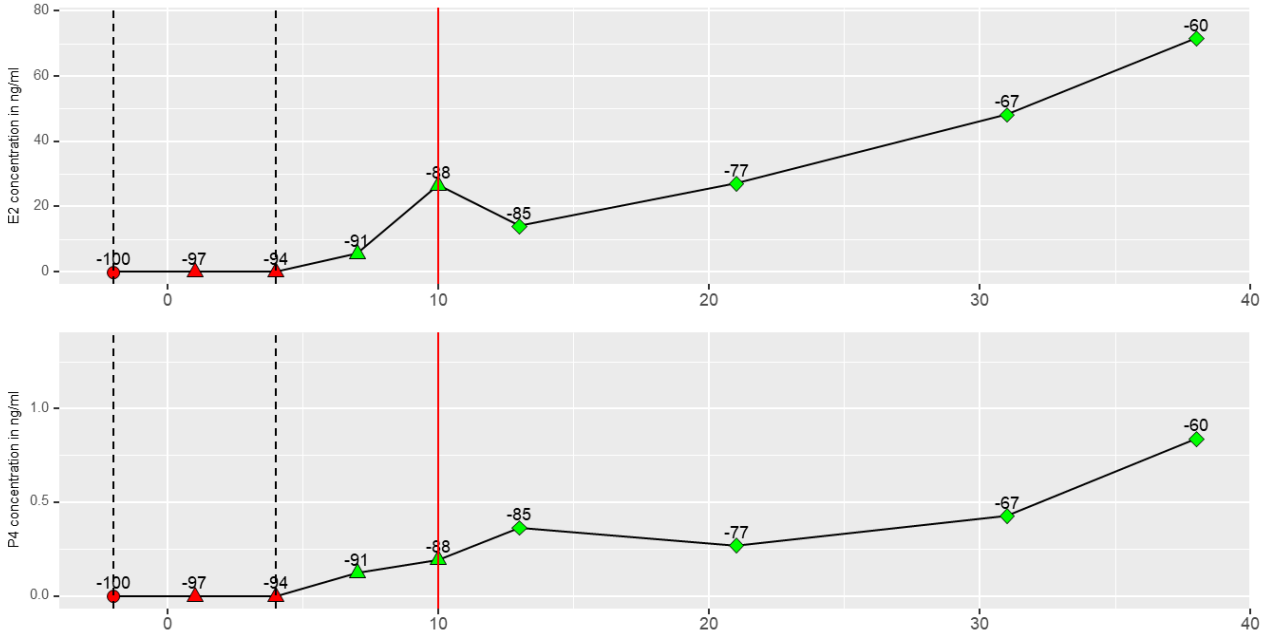
Unfamiliar Non-Kin: Jackass



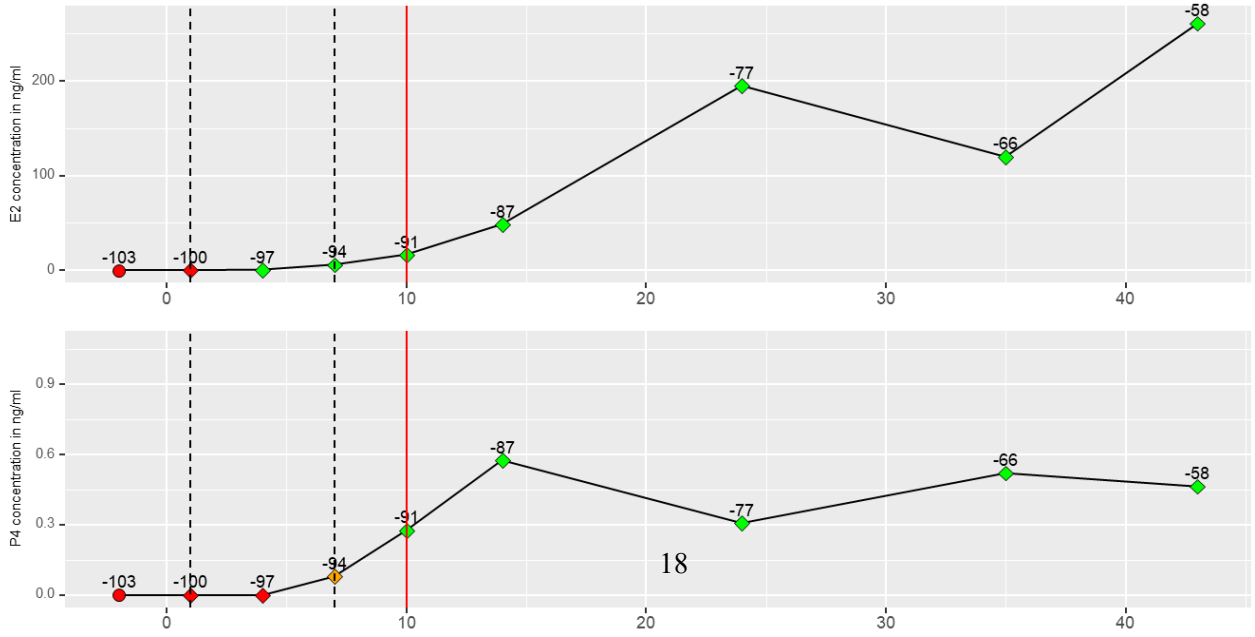
Unfamiliar Non-Kin: Kori

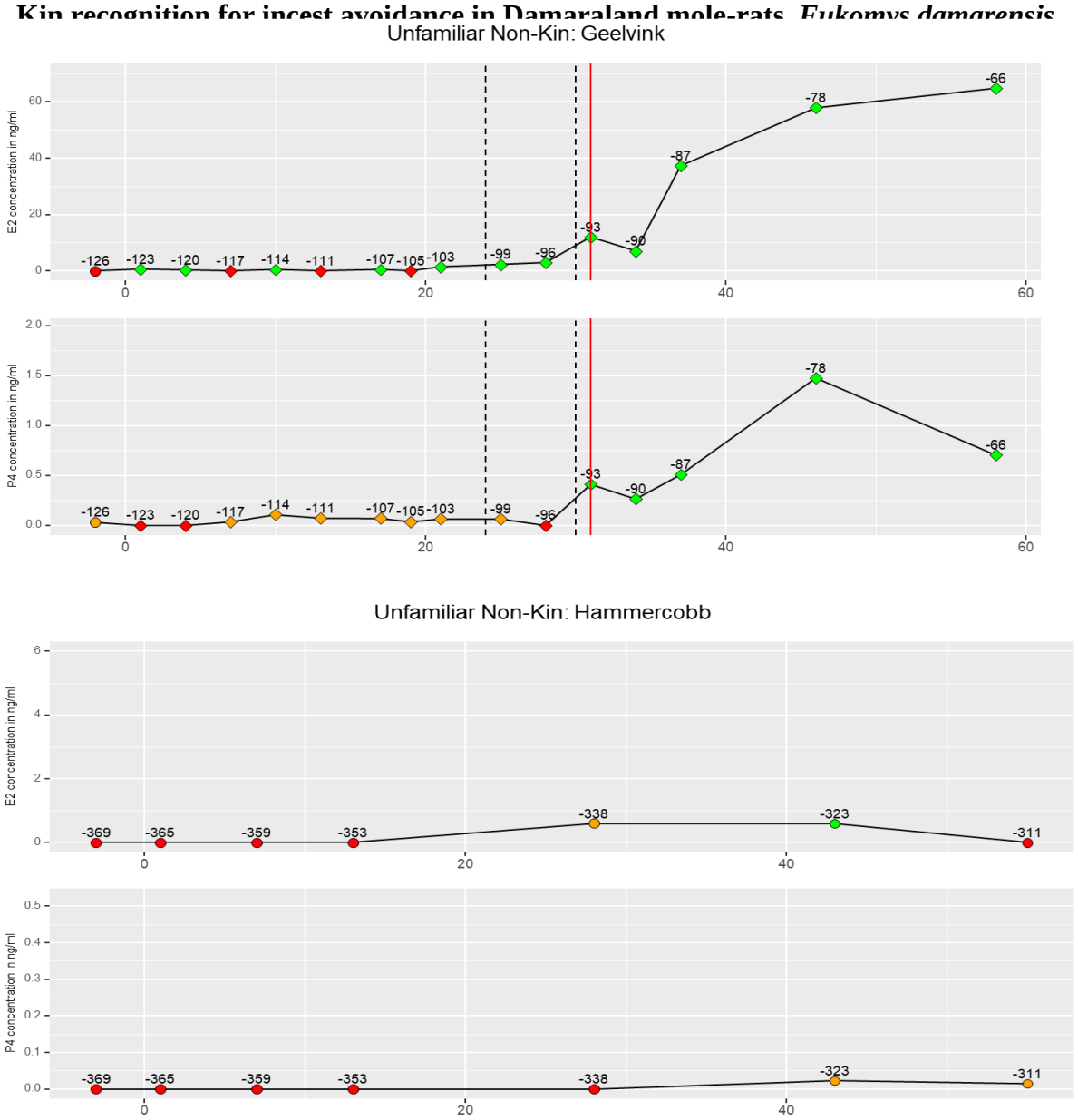


Unfamiliar Non-Kin: Marabou



Unfamiliar Non-Kin: Vulture





Figures S2D. Individual E2 and P4 profiles until 60 days post-pairing used for data exploration. Y-axis shows urinary concentrations of estradiol (E2) and progesterone (P4) in ng/ml. X-axis shows experimental day relative to pairing day. Red points indicate datapoints that were assigned a concentration of 0 because they fell below the limit of detection (LOD; signal/noise = 3), orange points indicate concentrations that fell between the LOD and the limit of quantification (LOQ; signal/noise = 8), green points indicate concentrations that fell above LOQ. Vertical red lines indicate the timing of reproductive activation determined by E2 levels of females that successfully produced a litter or showed a hormone profile suggestive of reproductive activation surpassing the highest E2 levels measured in other females (8.996 ng/ml, E2 criteria 1). Vertical blue lines indicate the end of a luteal phase or unsuccessful gestation. Vertical dashed line highlight a possible time window of successful mating (between 100 and 94 days post-pairing, unpublished data). For females that successfully produced a litter, the number of days prior to first parturition is indicated above each datapoint.

S3. Results: Behavioural observations

Table S3A. Outputs from tweedie GLMMs comparing the rate of sexual behaviours observed in female Damaraland mole-rats during two focal sessions, after being paired with a male that is either: familiar kin (n = 8); familiar non-kin (n = 8); unfamiliar kin (n = 7) or unfamiliar non-kin (n = 8). Female ID Session ID were included as random effects. Results are given on the log scale. The dispersion parameter of the tweedie distribution was estimated for each treatment, to account for differences in dispersion across treatments.

Behaviour	**Kinship**	**Estimate ± SE**	***z***	***p***
Copulation	Familiar kin; Intercept	-1.67 ± 1.04	-1.61	0.11
	Familiar non-kin	-0.02 ± 1.28	-0.02	0.99
	Unfamiliar kin	3.25 ± 1.02	3.19	<0.01
	Unfamiliar non-kin	3.20 ± 1.00	3.19	<0.01
Sex foreplay	Familiar kin; Intercept	-3.15 ± 0.94	-3.35	<0.001
	Familiar non-kin	2.07 ± 1.13	1.84	0.07
	Unfamiliar kin	5.02 ± 1.09	4.61	<0.001
	Unfamiliar non-kin	5.03 ± 1.07	4.70	<0.001

Table S3B. Pairwise comparisons of sexual behaviour observed by females during focal sessions (n = 31, df = 51). Post-hoc analyses are performed on the estimated marginal means computed from tweedie models with a log-link. Results are given on the log scale. P-values were adjusted using the Tukey method.

Behaviour	**Contrast**	**Estimate ± SE**	***t***	***p***
Copulation	Familiar kin - Familiar non-kin	0.02 ± .128	0.02	1.00
	Familiar kin - Unfamiliar kin	-3.25 ± 1.02	-3.19	<0.05
	Familiar kin - Unfamiliar non-kin	-3.20 ± 1.00	-3.19	<0.05
	Familiar non-kin - Unfamiliar kin	-3.27 ± 1.02	-3.21	<0.05
	Familiar non-kin - Unfamiliar non-kin	-3.23 ± 1.01	-3.21	<0.05
	Unfamiliar kin - Unfamiliar non-kin	0.04 ± 0.63	0.07	0.99
Sex foreplay	Familiar kin - Familiar non-kin	-2.07 ± 1.13	-1.84	0.27
	Familiar kin - Unfamiliar kin	-5.01 ± 1.09	-4.61	<0.001
	Familiar kin - Unfamiliar non-kin	-5.03 ± 1.07	-4.69	<0.001
	Familiar non-kin - Unfamiliar kin	-2.94 ± 0.95	-3.08	<0.05
	Familiar non-kin - Unfamiliar non-kin	-2.96 ± 0.94	-3.16	<0.05
	Unfamiliar kin - Unfamiliar non-kin	-0.02 ± 0.83	-0.02	1.00

Table S3C. Outputs from tweedie GLMMs comparing the rate of sexual behaviour observed during eight 12-hr scan sessions, after being placed in opposite-sex pairs of alternative relationship categories: familiar kin (n = 8); familiar non-kin (n = 8); unfamiliar kin (n = 7) or unfamiliar non-kin (n = 8). Pair ID and Session ID were included as random effects. Results are given on the log scale. The dispersion parameter of the tweedie distribution was estimated for each treatment, to account for differences in dispersion across treatments.

Behaviour	**Kinship**	**Estimate ± SE**	***z***	***p***
Copulation	Familiar kin; Intercept	-1.80 ± 0.67	-2.70	<0.01
	Familiar non-kin	-0.07 ± 0.84	-0.09	0.93
	Unfamiliar kin	2.76 ± 0.75	3.71	<0.001
	Unfamiliar non-kin	3.17 ± 0.72	4.44	<0.001
Sex foreplay	Familiar kin; Intercept	-1.97 ± 0.60	-3.27	<0.01
	Familiar non-kin	0.59 ± 0.75	0.80	0.43
	Unfamiliar kin	2.78 ± 0.69	4.05	<0.001
	Unfamiliar non-kin	3.31 ± 0.66	5.00	<0.001

Table S3D. Pairwise comparisons of sexual behaviour observed within opposite-sex pairs during scan sessions (n = 31, df = 485). Post-hoc analyses are performed on the estimated marginal means computed from tweedie models with a log-link. Results are given on the log scale. P-values were adjusted using the Tukey method.

Behaviour	**Contrast**	**Estimate ± SE**	***t***	***p***
Copulation	Familiar kin - Familiar non-kin	0.07 ± 0.84	0.09	0.99
	Familiar kin - Unfamiliar kin	-2.76 ± 0.75	-3.71	<0.01
	Familiar kin - Unfamiliar non-kin	-3.17 ± 0.72	-4.4	<0.001
	Familiar non-kin - Unfamiliar kin	-2.84 ± 0.71	-4.00	<0.001
	Familiar non-kin - Unfamiliar non-kin	-3.25 ± 0.68	-4.77	<0.001
	Unfamiliar kin - Unfamiliar non-kin	-0.41 ± 0.51	-0.80	0.85
Sex foreplay	Familiar kin - Familiar non-kin	-0.59 ± 0.75	-0.80	0.86
	Familiar kin - Unfamiliar kin	-2.78 ± 0.69	-4.05	<0.001
	Familiar kin - Unfamiliar non-kin	-3.31 ± 0.66	-4.99	<0.001
	Familiar non-kin - Unfamiliar kin	-2.18 ± 0.64	-3.42	<0.01
	Familiar non-kin - Unfamiliar non-kin	-2.72 ± 0.61	-4.45	<0.001
	Unfamiliar kin - Unfamiliar non-kin	-0.53 ± 0.52	-1.03	0.73

S4. Results: Reproductive physiology

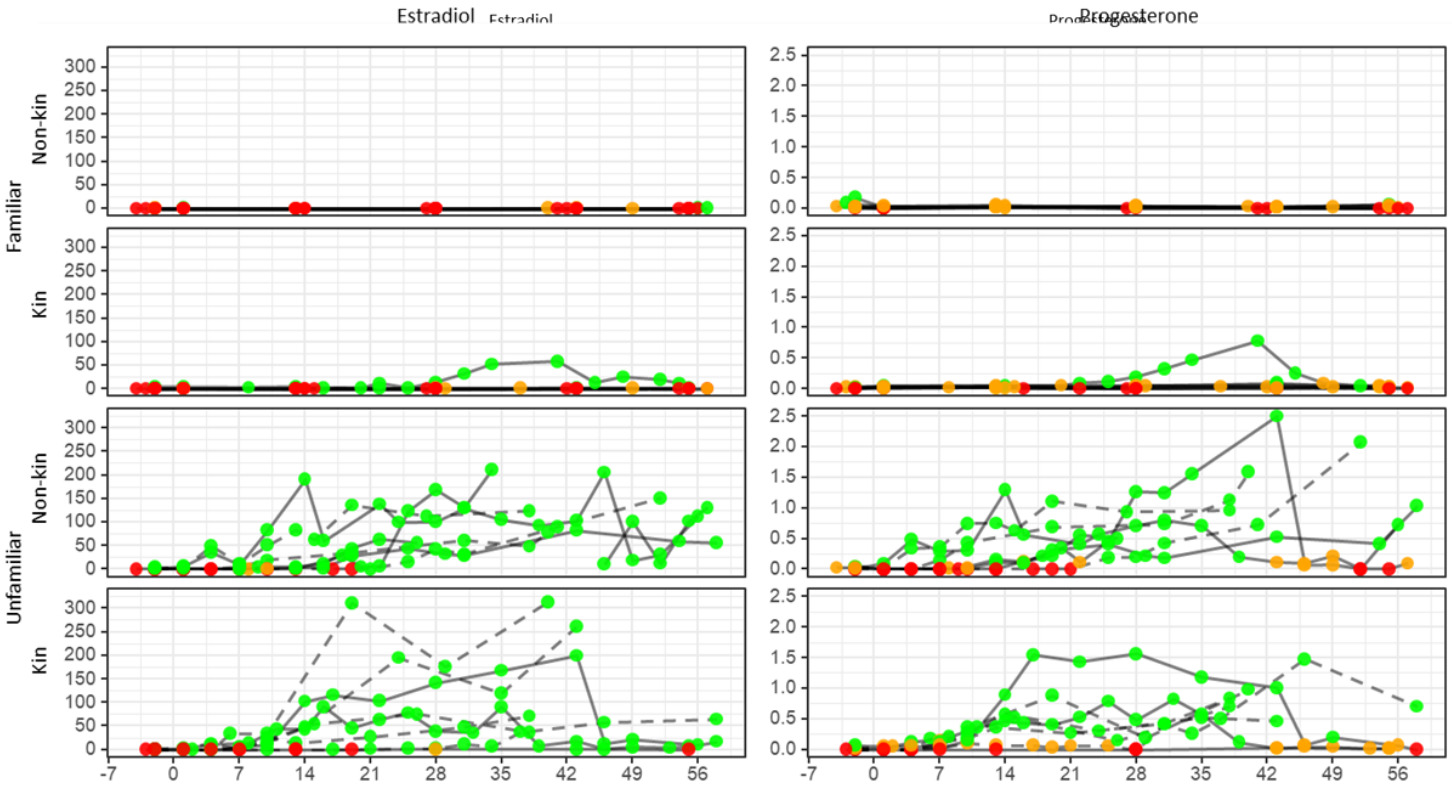


Figure S4A. E2 and P4 profiles of females paired with familiar kin, familiar non-kin, unfamiliar kin and unfamiliar non-kin males. Y-axis shows urinary concentrations of estradiol (left column) and progesterone (right column) in ng/ml. X-axis shows experimental day relative to pairing day. Lines indicate repeated measures of same individuals. Solid lines indicate hormone profiles for which reproductive activation was associated with a successful gestation whereas dotted lines indicate hormone profiles for which reproductive activation was associated with an interrupted gestation or a luteal phase. Red points indicate datapoints that were assigned a concentration of 0 because they fell below the limit of detection (LOD; signal/noise = 3), orange points indicate concentrations that fell between the LOD and the limit of quantification (LOQ; signal/noise = 8), green points indicate concentrations that fell above LOQ.

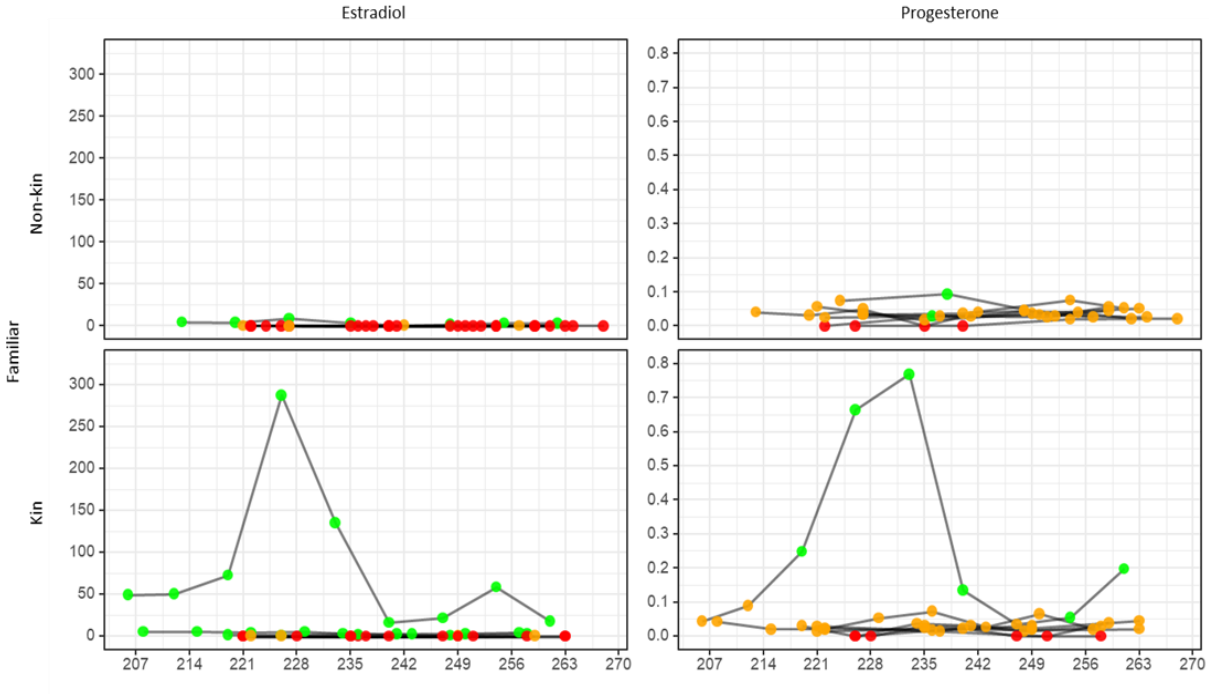


Figure S4B. E2 and P4 profiles of females paired with familiar males at the end of the experiment. Y-axis shows urinary concentrations of estradiol (left column) and progesterone (right column) in ng/ml. X-axis shows experimental day relative to pairing day. Lines indicate repeated measures of same individuals. Red points indicate datapoints that were assigned a concentration of 0 because they fell below the limit of detection (LOD; signal/noise = 3), orange points indicate concentrations that fell between the LOD and the limit of quantification (LOQ; signal/noise = 8), green points indicate concentrations that fell above LOQ.

Table S4C: Outputs from tweedie GLMMs with a log-link comparing the levels of estradiol and progesterone measured in female Damaraland mole-rats, after being placed in pairs of alternative relationship categories: familiar kin (n = 8); familiar non-kin (n = 8); unfamiliar kin (n = 7) or unfamiliar non-kin (n = 8). Female ID was included as a random effect in all models. Results are given on the log scale.

Hormone	**Treatment**	**Estimate ± SE**	***z***	***p***
Estradiol	Familiar kin; Intercept	-2.72 ± 0.75	-3.64	<0.001
	Familiar non-kin	2.29 ± 0.98	2.34	0.02
	Unfamiliar kin	6.54 ± 0.88	7.42	<0.001
	Unfamiliar non-kin	6.10 ± 0.87	7.04	<0.001
Progesterone	Familiar kin; Intercept	-1.98 ± 0.27	-7.45	<0.001
	Familiar non-kin	0.30 ± 0.26	1.16	0.25
	Unfamiliar kin	1.76 ± 0.25	7.12	<0.001
	Unfamiliar non-kin	1.46 ± 0.24	6.07	<0.001

Table S4D. Pairwise comparisons of post-pairing hormone levels (n = 31, df = 252). Post-hoc analyses were performed on the estimated marginal means computed from tweedie models with a log-link. Results are given on the log scale. P-values were adjusted using the Tukey method.

Hormone	**Contrast**	**Estimate ± SE**	***t***	***p***
Estradiol	Familiar kin - Familiar non-kin	-2.29 ± 0.98	-2.34	0.09
	Familiar kin - Unfamiliar kin	-6.55 ± 0.88	-7.42	<0.001
	Familiar kin - Unfamiliar non-kin	-6.10 ± 0.87	-7.04	<0.001
	Familiar non-kin - Unfamiliar kin	-4.25 ± 0.84	-5.03	<0.001
	Familiar non-kin - Unfamiliar non-kin	-3.81 ± 0.81	-4.71	<0.001
	Unfamiliar kin - Unfamiliar non-kin	0.44± 0.66	0.68	0.91
Progesterone	Familiar kin - Familiar non-kin	-0.58 ± 0.36	-1.60	0.38
	Familiar kin - Unfamiliar kin	-2.99 ± 0.35	-8.53	<0.001
	Familiar kin - Unfamiliar non-kin	-2.62 ± 0.34	-7.61	<0.001
	Familiar non-kin - Unfamiliar kin	-2.41 ± 0.35	-6.85	<0.001
	Familiar non-kin - Unfamiliar non-kin	-2.04 ± 0.21	-0.34	<0.001
	Unfamiliar kin - Unfamiliar non-kin	0.37 ± 0.34	-1.10	0.69

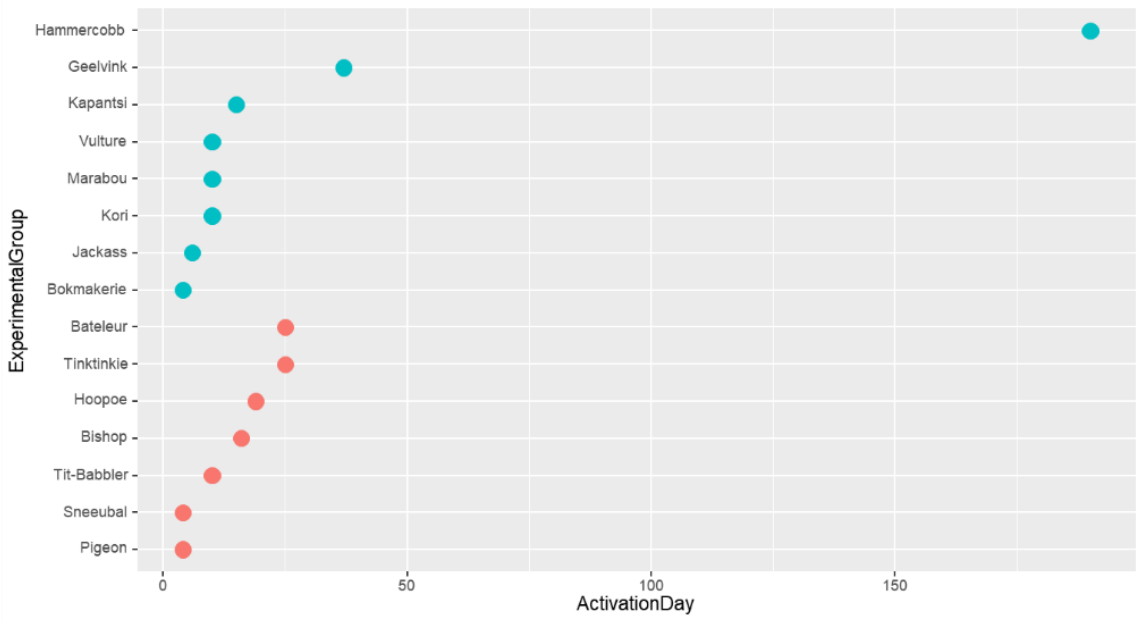


Figure S4E. Reproductive activation in days post-pairing of females paired with an unfamiliar male. Blue dots show datapoint from females paired with non-kin (n = 8). Red dots show datapoints from females paired with kin (n = 7). Reproductive activation is here determined using the second defining criteria of reproductive activation (earliest post-pairing day when E2 levels surpassed 8.996ng/ml in two successive samples).

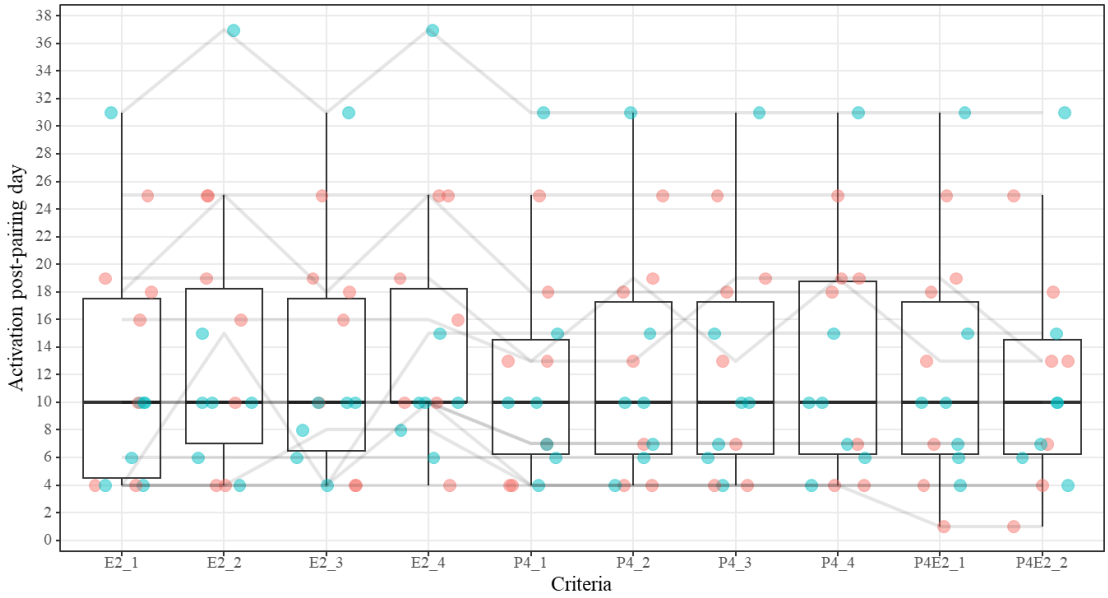


Figure S4F. Timing of reproductive axis activation, expressed in number of days post-pairing, for each of the criteria upon which reproductive activation was defined. Red dots show females paired with unfamiliar kin (*n* = 7). Blue dots show females paired with unfamiliar non-kin (*n* = 7, most extreme outlier removed). The most extreme outlier from the unfamiliar non-kin treatment (Hammercobb) is not shown. Lines indicate repeated datapoints of individuals. Horizontal jitter was added to improve visualization.

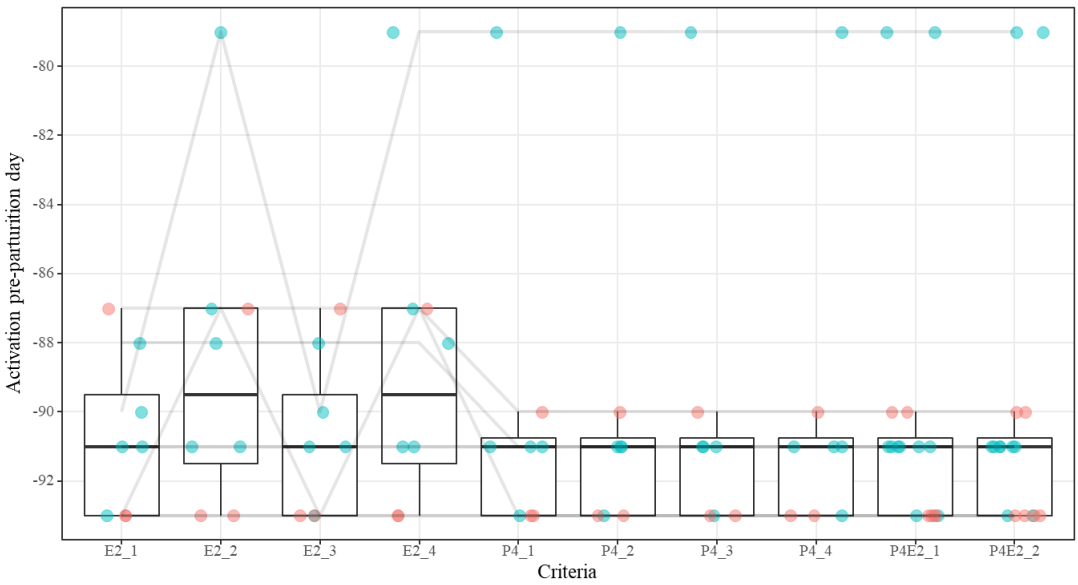


Figure S4G. Timing of reproductive axis activation, expressed in number of days prior to first parturition, in females with a successful gestation (production of litter within 100 days of activation). Red dots show females paired with unfamiliar kin. Blue dots show females paired with unfamiliar non-kin. Lines indicate repeated datapoints of individuals. Horizontal jitter was added to improve the visualization of individuals with similar activation day.

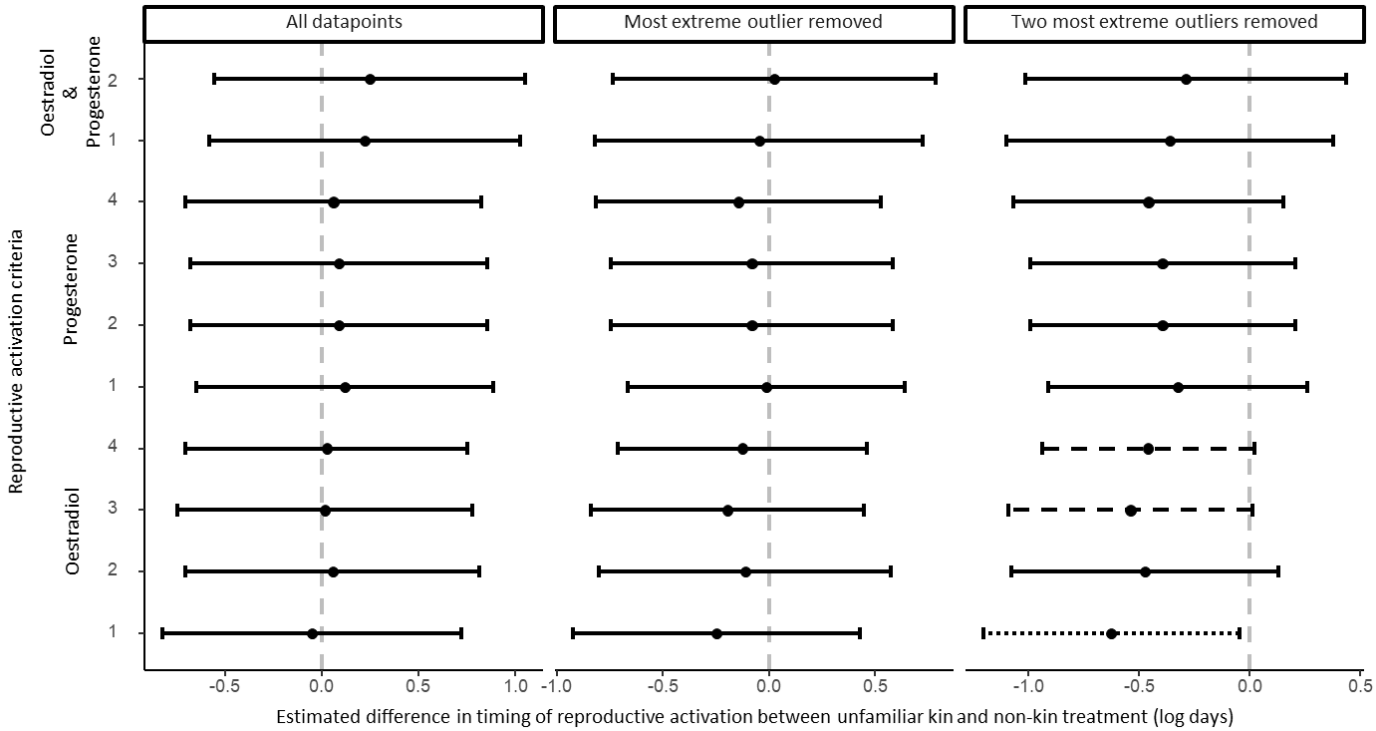


Figure S4H. Differences in the timing of reproductive activation between females paired with unfamiliar kin and unfamiliar non-kin (n = 31). Dots indicate the differences in timing of reproductive activation estimated by a generalized poisson (all data) and gamma (outliers removed) GLMMs with log link, and expressed as the log(days) of reproductive activation in kin pairs minus the log(days) of reproductive activation in non-kin pairs. Models were separately fitted based on (i) the ten criteria used to determine reproductive activation (see Methods and Supplementary Material S2) and (ii) management of outliers (no outlier removed, most extreme outlier removed, two outliers removed). Horizontal lines represent confidence intervals of model estimates. Solid line indicates a p-value > 0.1, dashed line a p-value between 0.05 and 0.1 and dotted lines a p-value <0.05.

S5. Results: Reproductive success

Table S5A: Outputs from Poisson GLMM comparing the number of pups produced by females that successfully mated with; (i) unfamiliar kin and (ii) and unfamiliar non-kin.

Variable	**Treatment**	**Estimate ± SE**	***z***	***p***
Number of pups	Unfamiliar kin; Intercept	1.75 ± 0.25	6.90	<0.001
	Unfamiliar non-kin	0.02 ± 0.33	0.08	0.94
Number of litters	Unfamiliar kin; Intercept	0.83 ± 0.25	3.31	<0.001
	Unfamiliar non-kin	-0.02 ± 0.34	-0.05	0.96

Table S5B: Outputs from binomial GLM with a log-link estimating the likelihood of reproductive activation being associated with a successful gestation, i.e. successful gestation occurring within 100 days of reproductive activation. Results are given on the log scale.

Successful gestation (yes/no)	**Treatment**	**Estimate ± SE**	***z***	***p***
	Unfamiliar kin; Intercept	0.29 ± 0.76	0.38	0.71
	Unfamiliar non-kin	1.20 ± 1.13	1.06	0.29