# Fecal genotyping to estimate small mammal population size, with a comparison to live mark-recapture estimates

March 28, 2024

## FULL RESEARCH ARTICLE

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Published 28 March 2024 • doi.org/10.51492/cfwj.110.1

# Abstract

Live capture-recapture is often considered the gold standard for estimating wildlife population size or density, but the approach can be limited by permitting requirements, required labor, welfare concerns, and biased estimates resulting from heterogeneity in individual behavior. Noninvasive genetic sampling (e.g., from fecal pellets) offers a powerful alternative approach, but this method's success varies among taxa, with little research available on its use in rodents. Here, we addressed a series of questions to develop a noninvasive genetic sampling approach for the endangered giant kangaroo rat (*Dipodomys ingens*): (1) how quickly does DNA degrade in natural conditions, (2) how many pellets are required to recover a genotype, (3) how often do multiple individuals contaminate a pooled sample from a single sampling location, and (4) how do variable and parameter estimates from noninvasive genetic sampling compare to live-trapping mark-recapture estimates? We found that fecal pellets were successfully genotyped up to 9 days (estimated probability of recovery = 0.78) after exposure to hot, arid conditions, but that rate fell precipitously soon after. Although giant kangaroo rats are territorial, multiple individuals deposited fecal pellets at the same sampling locations; however, single pellets contained sufficient DNA

to recover genotypes and to identify individuals, so contamination was not a problem for this approach. Capture probabilities were lower using noninvasive genetic sampling ( = 0.26, SE = 0.01) than live trapping ( = 0.40, SE = 0.06). Population estimates were generally similar using noninvasive genetic sampling, although they were quite a bit higher ( = 64 , = 38) on one grid. Noninvasive genetic sampling can overcome many of the limitations of live-trapping for small mammals, but the approach should be tested in additional taxa and systems to provide more generalizable recommendations for sampling schemes.

**Key words:** capture-recapture, California, *Dipodomys ingens*, DNA,fecal pellets, giant kangaroo rats, live trapping, noninvasive, San Joaquin Desert

**Citation:** Bean, W. T., M. J. Statham, M. Treiber, W. B. Claflin, C. M. Fiehler, and B. N. Sacks. 2024. Fecal genotyping to estimate small mammal population size, with a comparison to live mark-recapture estimates. California Fish and Wildlife Journal 110:e1.

**Editor:** Grace Myers, Habitat Conservation Planning Branch

**Submitted:** 13 September 2023; **Accepted:** 13 November 2023



**Funding:** Funding was provided through a Section 6 grant from U.S. Fish and Wildlife Service, the William and Linda Frost Fund from the Bailey College of Science and Math at Cal Poly SLO, and in-kind support from California Department of Fish and Wildlife and Cal Poly SLO.

**Competing Interests:** The authors have not declared any competing interests.

# Introduction

Population abundance estimation is essential to conservation and management, both for establishing species status and for assessing the effectiveness of management practices. While mark-recapture is often considered the gold standard for abundance estimation, multiple challenges with this approach remain. First, obtaining estimates can be difficult and time-intensive (Conroy et al. 2008). Next, live capture often requires a substantial permitting process, particularly for special status species (Sikes and Paul 2013). Third, mark-recapture typically relies on the assumption that all individuals in a population share equal probability of capture. However, studies of capture probability in small mammals often indicate that some individuals may be trap-avoidant, leading to an over-estimate of capture probability and an under-estimate of population size (e.g., Hammond and Anthony 2008). In extreme cases, some species of small mammal may be difficult to nearly impossible to capture in live traps (e.g., white-footed voles *Arborimus albipes*;Maser and Johnson 1967). Finally, traditional mark-recapture poses welfare concerns given the risks of stress, injury, or mortality inherent to live trapping (Putman 1995; Zemanova 2020).

Emerging population monitoring techniques aim to resolve the limitations of mark-recapture methods while still providing reliable and precise population estimates. One such alternative is capture-recapture

estimation from noninvasive genetic sampling, a relatively novel method allowed for by advances in molecular technology (Waits and Paetkau 2005). Noninvasive genetic sampling utilizes DNA extracted from fur, saliva, feces, or other sources to obtain individual identification (Ferreira et al. 2018). This method has the potential to reduce field expenses and bureaucratic burdens related to live handling, eliminate welfare risks, as well as provide insight into individual differences in trap-capture probabilities that may bias traditional mark-recapture estimates. Noninvasive genetic sampling has been widely implemented, primarily in population estimates for large, wide-ranging carnivores (Mondol et al. 2009; Stenglein et al. 2010; Owen-Ramos et al. 2022) or ungulates (Henk et al. 2022; Batter et al. 2022). The technique has also been used for lagomorphs (DeMay et al. 2017; Uhrig 2019; Bauer et al. 2020). While there is the potential for broad application in rodents, we are aware of only one other study that has employed noninvasive genetic sampling for this purpose. Ferreira et al. (2018) tested a large-scale approach to monitor a metapopulation of the Cabrera vole (*Microtus cabrerae*) in Iberia in winter and identified more than twice as many individuals from the noninvasive sampling compared to live trapping. Further research is therefore needed in other biomes, seasons, and taxa to develop a generalizable technique for small mammals, given the potential differences in spatial scale, behavior, and amount of DNA available and degradation rate which may require samples of multiple pellets to resolve individual genotypes.

While noninvasive genetic sampling can alleviate many concerns related to traditional mark-recapture approaches, it is not without potential problems. Common issues include scarcity of DNA in individual fecal pellets and sample degradation, resulting in an inadequate amount of genetic material to resolve to genotype (Waits and Paetkau 2005). An additional concern is contamination. Multiple pellets may be required to amplify sufficient DNA, but all pellets must be from the same individual to resolve a single genotype. For small mammals, it may also be unclear to what extent individual home ranges overlap and therefore how to design a sampling scheme to minimize the collection of pellets from multiple individuals in the same sample.

Challenges related to mark-recapture estimates from live trapping are especially relevant for the giant kangaroo rat (GKR, *Dipodomys ingens*), a federally- and state-endangered species, as a result of increased permit and training requirements. The GKR is a nocturnal, burrowing rodent endemic to the San Joaquin Desert, California, which subsists almost entirely on seeds for food and water, and whose historical range has been vastly reduced due to agricultural expansion (Williams and Kilburn 1991). Giant kangaroo rats are generally territorial, particularly during the summer non-breeding season, but home ranges may overlap at the edges (Cooper and Randal 2007). The species is competitively dominant to other nocturnal rodents, resulting in their near exclusion from core parts of GKR home ranges (Prugh et al. 2018). The only other small mammal occurring regularly in the study area was the San Joaquin antelope squirrel (*Ammospermophilus nelsoni*), for which fecal pellets are readily distinguished as larger and lighter in color than those of kangaroo rats.

Here, we assess the use of noninvasive genetic sampling for abundance estimates of GKR and attempt to resolve four key questions to develop a robust technique for deploying this method for small mammals more broadly. Specifically, we conducted live mark-recapture trapping and noninvasive fecal pellet genetic sampling to resolve these questions: (1) how quickly does genetic material in fecal pellets degrade when exposed to the elements, (2) how many pellets are required to recover a genotype, (3) how frequently do multiple individuals overlap to contaminate a pooled sample from the same location and (4) how do population estimates from noninvasive genetic sampling compare to live mark-recapture estimates?

# Methods

The Carrizo Plain National Monument is located in eastern San Luis Obispo County, CA (35.110, –119.733). Mean annual precipitation was approximately 25 cm, falling almost exclusively as winter rain between December and March (WRCC 2013). Vegetation on the Monument was dominated by red brome (*Bromus rubens*) and other non-native annual grasses with loamy and clay-loamy soils. Mean maximum daily temperature in the study area in July and August 2020–2021 was 31°C, mean minimum temperature was 20°C, and mean daily temperature was 27°C; there was no precipitation in these months (WRCC 2013).

In the summers of 2020 and 2021, we assessed noninvasive genetic sampling of giant kangaroo rats in the Carrizo Plain National Monument (**Fig. 1**). We conducted live-trapping and fecal monitoring on 30 grids that were monitored as part of a broader long-term monitoring project (Carrizo Plain Ecosystem Project [Prugh and Brashares 2012]). On one of the grids ("C1") in 2020, we also collected genetic samples from live-trapped individuals. One week after live-trapping concluded, we collected freshly deposited fecal pellets from one ("C1", 2020) or two ("C1" and "E1", 2021) grids. We conducted an exposure experiment to test the rate at which DNA in fecal pellets degraded in this environment.

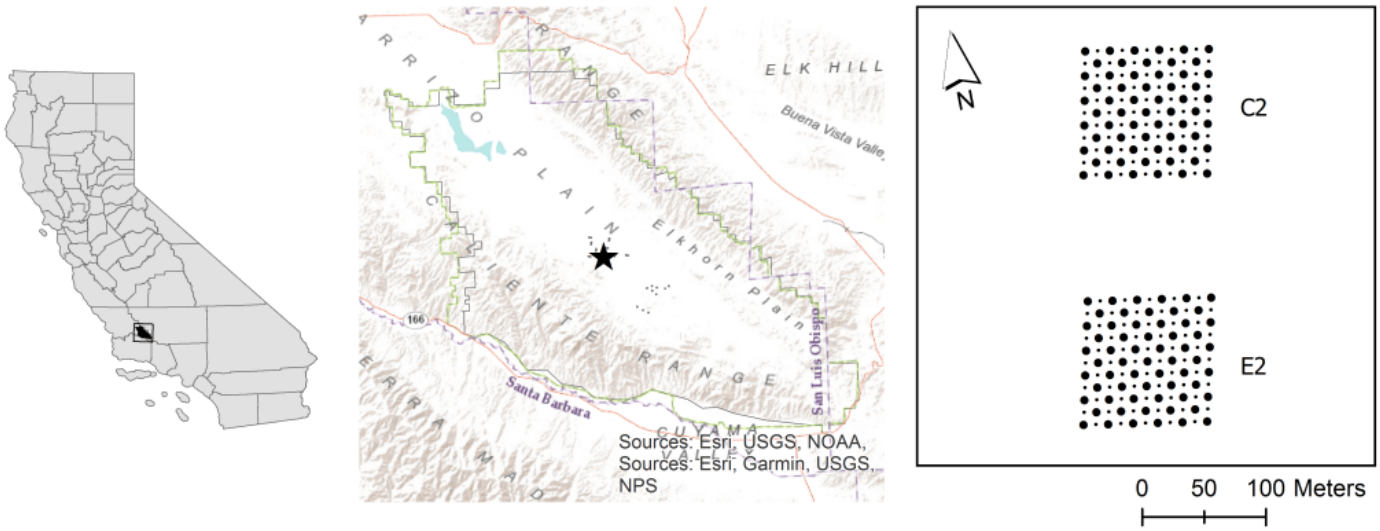


**Figure 1**. The main study area for giant kangaroo rats (2020–2011) consisted of two grids in the long-term Carrizo Plains Ecosystem Project, located in the Carrizo Plain National Monument, San Luis Obispo County, California. Both plots were sampled in an 11 x 11-point grid (10 m spacing), with live trapping occurring on every other point in a checkerboard pattern (larger circles), and fecal pellet collection occurring at every point (larger and smaller circles).

## Live Trapping

Following protocols from the Carrizo Plain Ecoystem Project, we set traps in a 100 m x 100 m checkerboard, with traps set 20 m apart for a total of 60 or 61 traps per grid, depending on whether the center trap was removed (grids with a rodent exclosure) (Prugh and Brashares 2012) (**Fig. 1**). Traps were opened at sunset, baited with bird seed, primarily white millet, and a paper towel, and checked approximately three to five hours later. Captured individuals were measured following standard small mammal protocols and received one Monel #1 ear tag in each ear (National Band & Tag Company, Newport, KY, USA). On one grid ("C2") in 2020, we also collected a tissue sample from the ear and, if

available, a fresh fecal pellet from each individual (n= 22) for genotyping. Using sterilized scissors, we cut a small sliver of tissue (~1 mm x 2 mm) from the tip of the ear and placed it into a 2-ml microcentrifuge tube containing 1 ml of >95% ethanol. Between sampling events, scissors were dipped in a 2% bleach solution, rinsed with water, dried with a Kimwipe, and stored in a sterile container.

## Noninvasive Genetic Sampling

We collected freshly deposited fecal pellets from one grid in 2020 (“C2”) and two grids in 2021 (“C2” and “E2”) (**Fig. 1**). One week after live trapping these grids, we established noninvasive sample locations at 10-m intervals, aligned with the live trapping grid, but at each grid location (i.e., 120 or 121 points) rather than a checkerboard. We did this to better understand the spatial pattern of fecal deposition within an individual kangaroo rat’s home range. Each point was marked by a labeled stake in the ground. At dusk, we swept clear a 1-$m^2$ area around each stake and baited the center with 29.6 ml (i.e., 1/8 cup) of millet. The following morning, we collected all fresh fecal pellets found at each stake using sterile gloves, and we placed them into coin envelopes. All fecal pellets found at a single stake were then placed together into a Ziploc bag containing 1.85 ml (1/8 Tbsp) of Sorbead Orange desiccant. In 2020, we collected all pellets from the first three rows of the grid regardless of “freshness; ” after these three rows were completed, we collected only pellets that were considered recent (smooth, uncracked, dark in color) and ignored older pellets (hard with a wrinkled, desiccated appearance, cracked, dull gray in color). Stakes were re-swept that evening and the collection process repeated the following morning. In 2020, we collected pellets for three consecutive days, and in 2021 we collected pellets for five consecutive days. In 2020, we sampled for three days to mimic live-trapping; live-trapping on the Carrizo Plain Ecosystem Project was initially conducted over five nights, but higher capture success allowed accurate population estimation with just three nights. After 2020, due to lower “capture” success from the fecal pellets, we expanded sampling for an additional two days.

## Genetic Analysis

We extracted DNA from fecal samples using 96 well DNA extraction kits. For samples from 2020, we used a Purelink Pro 96 Genomic DNA kit (Invitrogen, Waltham, MA, USA), for samples from 2021, we used a DNeasy 96 Blood & Tissue Kit (Qiagen, Germantown, MD, USA). In each case we modified the standard protocol to improve extraction from kangaroo rat pellets. In 2020, for each sample we used ≤3 pellets homogenized in 1.4 ml of buffer ASL (Qiagen, Germantown, MD, USA). We subsequently found that 1 pellet was sufficient for genotyping; therefore in 2021, we used single fecal pellets homogenized in 1.3 ml of buffer ASL. We centrifuged the samples to pellet the particulate matter. We added 1 ml of the supernatant to ~0.75 ml of potato starch and vortexed vigorously for 1 min. Following centrifugation, we transferred 300 µl of the supernatant to a 96 well block from the extraction kit, along with 20 µl of proteinase K. Subsequently we followed the manufacturer’s protocols. We extracted DNA from tissue samples using a DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA).

We screened tissues and ground-collected fecal samples at 11 microsatellite loci (dst119, dst1318, dst168, dst2601, dst2887, dst2793, dst1210, dst1230, dst1567, dst3268, dst3315; Shier et al. 2021) previously found to perform well in *D. ingens* (Statham et al. 2019). We PCR-amplified the loci in 2 multiplex reactions containing 6 and 5 loci respectively using the Qiagen multiplex kit according to the manufacturers recommended protocols (including Q-solution). The fecal samples used in the exposure time trial were collected during live-capture from known animals, where the objective was to assess DNA

degradation over time (rather than to identify samples to individual), therefore the samples were only genotyped at the first multiplex of six loci. We amplified PCR products following the PCR mixture and thermal profile of Statham et al. (2019). We electrophoresed products on an ABI 3730 capillary sequencer and scored alleles relative to an internal size standard, Genescan 500 LIZ (Applied Biosystems), using STRand software (Locke et al. 2007). All ground collected fecal samples were genotyped in duplicate. We did not run PCR replication on the exposure time trial fecal samples.

We used the genotype data to identify individual kangaroo rats. For the purposes of genetic mark recapture population size estimation, we wanted to identify when the same individuals were recaptured. When genotypes from two independent samples matched 100%, this was considered a recapture of the same individual. However, fecal samples are prone to genotyping errors, meaning that samples originating from the same individual may result in genotypes that do not 100% match due to allelic dropout (Prugh et al. 2005). Therefore, we needed to determine a cutoff for very similar genotypes to be assigned as the same individual. We compared the percent allele sharing among genotypes to assess if there was a natural break between distinct individuals versus genotypes originating from the same individual.

*How quickly does genetic material in fecal pellets degrade when exposed to the elements?*

To determine fecal degradation rates, we first collected fresh fecal pellets directly from the live-capture handling bags. Then, we divided these fresh pellets into groups which were subsequently exposed in natural conditions to test their effects on genotyping success rates. Pellets were placed directly on soil typical of the Carrizo Plain in summer months. The samples were placed in full sun, 15 cm apart and within a 10 cm tall cardboard windscreen measuring 50 cm x 50 cm to prevent loss or mixing. In 2020, samples were placed on 27 July and collected after 0.5, 1, 3, 5, 7, and 9 days. Each timed exposure had three pellets, except the 9-day sample which had only two. We also tested pellets collected at the time of live trapping and used to genotype individuals in the traps (n = 27), treating these as having been exposed for 0 days. In 2021, we placed samples on 5 August and collected them after 10, 30, and 60 days (n = 12, 10, 10, respectively). After the appropriate exposure time, we placed samples into a coin envelope, which was placed in a Ziploc bag with Sorbead Orange desiccant (Delta Adsorbents, Roselle, IL, USA). Samples were stored at room temperature for 3–5 months until DNA extraction.

We analyzed the degradation rate of DNA in these fecal pellets through exposure duration. For each sample, we attempted to amplify six loci and categorized the results as resolving a full genotype when ≥5 of six loci amplified. Then, we estimated the impact of time on the probability of recovering a genotype using a simple logistic regression, with genotype recovery (0 or 1) as the response variable and time exposed as the predictor.

*How many pellets are required to resolve to genotype?*

In 2020, we genotyped 1–3 pellets at a time to estimate how many were needed to obtain sufficient DNA. We extracted DNA from one and three pellets, with seven samples of each. All samples were from live trapped individuals. In addition, we genotyped pooled samples and calculated the percent of each that recovered a genotype.

*How frequently do multiple individuals overlap to contaminate a single sample from the same location?*

We identified sample stakes which had multiple genotypes in the fecal pellets. In 2020, some pellets were pooled for processing, and of these, some had mixed genotypes as a result; however, we also found that one pellet was sufficient for genotyping. Therefore, in 2021, each fecal pellet collected was processed individually. We therefore report both the mixed genotype samples as well as how often multiple genotyped fecal pellets were found at the same stake.

*How do population estimates from noninvasive genetic sampling compare to live mark-recapture estimates?*

We separately estimated population size using the live trapping data and the fecal pellet collection. For each grid and year ("C2" for 2020 and "C2" and "E2" for 2021), we estimated capture probability and population size using the Huggins model (Lukacs 2021) with package "mra" (McDonald 2018). Fecal pellets were collected at more stations (i.e., at every point on a 12 x 12 grid) than the live trapping (every other point in checkerboard fashion) So, to produce a more equitable comparison, we also estimated population size and capture probability from the noninvasive genetic sampling approach using only fecal pellets found at stakes where live traps had been placed. Then, for each grid, session, and modeling approach, we compared the estimates from live trapping and non-invasive genetic sampling.

# Results

## Live Trapping

In 2020, we captured 28 individuals on grid "C2": eight on night 1, six on night 2 (two recaptures), and 14 on night 3 (five recaptures) and collected tissue samples from all (**Fig. 2**). In 2021, we captured 25 individuals on grid "C2": 18 on night 1, 11 on night 2 (two recaptures), and 9 on night 3 (four recaptures). We captured 37 individuals on grid "E2": 12 on night 1, 16 on night 2 (four recaptures), and 17 on night 3 (four recaptures).

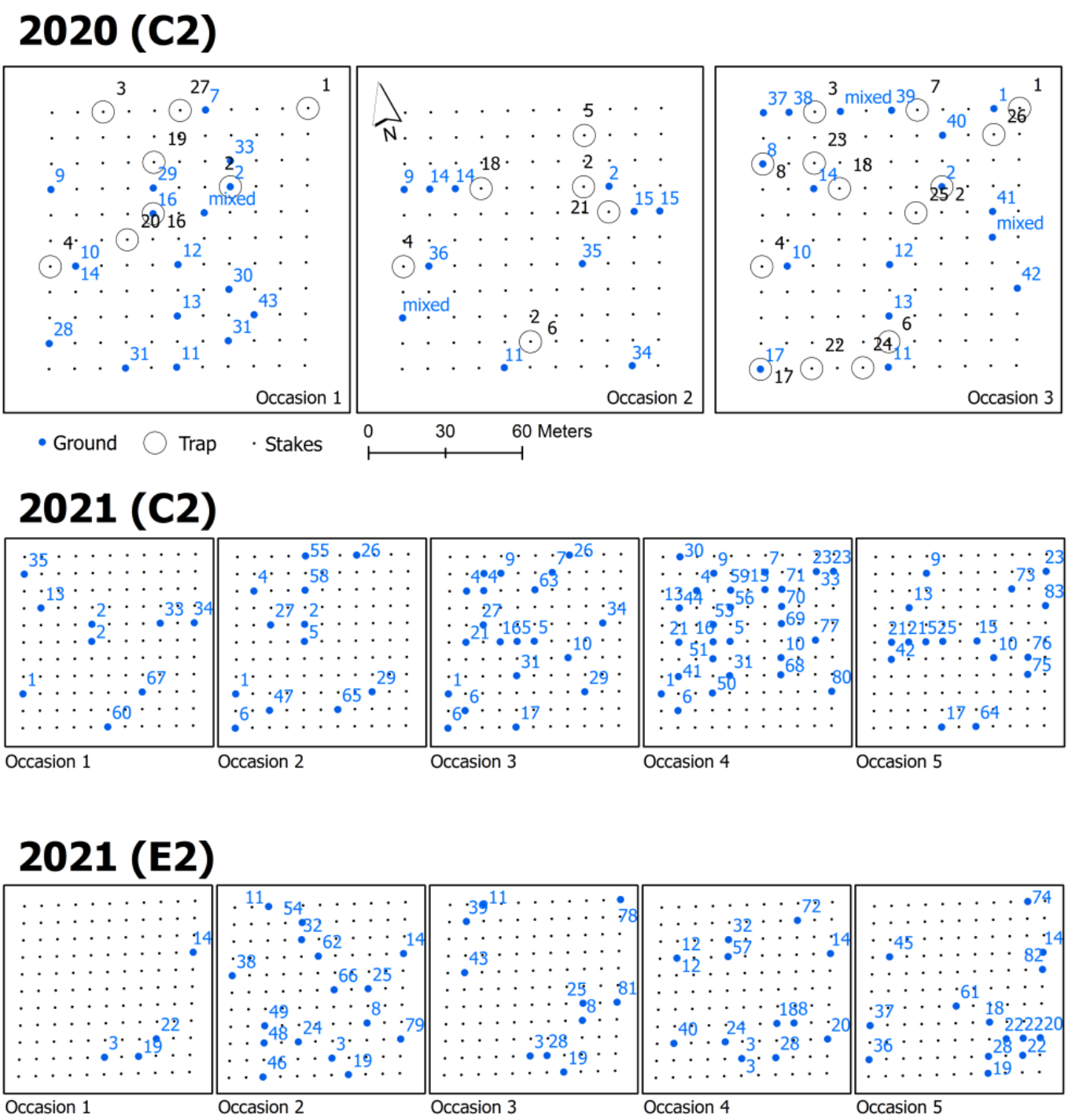


**Figure 2**. Locations of genotyped giant kangaroo rats across occasions, grids, and years (2020–2021), Carrizo Plain, California. Black dots represent stakes used to sample fecal pellets. Individuals genotyped from fecal pellets shown in blue, individuals genotyped from live traps (2020 C2 only) as black circles. Genotype IDs are unique to year.

## Noninvasive Genetic Sampling and Genetic Analysis

The microsatellite markers were highly polymorphic with 5–21 alleles per locus (**SI Table 1 (PDF)**). The probability of identity (PID) and probability of identity of siblings (PIDsibs) were $3.5 \times 10^{-15}$ and $1.9 \times 10^{-5}$ respectively, thus providing a very high resolving power to identify individuals. We compared the percent allele sharing among genotypes collected from all samples and used this information to assess the relationship among individuals, and to determine a cutoff for genotypes being assigned to the same

individual. The results were bimodal, with most samples sharing between 0–70% of their alleles, and a smaller secondary peak of 89–100% (**SI Fig. 1 (PDF)**). Based on this information we considered samples with genotypes matching ≥89% as originating from the same individual, which equated to allowing differences at up to two loci between genotypes. In the majority of cases these differences could be explained by dropout of one of two alleles in a heterozygous locus.

We collected 109 fecal samples on C2 in 2020, 112 on C2 in 2021, and 82 on E2 in 2021 (**Table 1**). Of those, 41 (38%), 87 (78%) and 58 (62%) resulted in an identifiable genotype. These differences were statistically different among years ($\chi_2^2 = 37.9$, $P < 0.001$).

**Table 1.** Summary of number of giant kangaroo rat fecal pellets collected by grid and year (2020–2021). Individual pellets were pooled into a single sample from a 1-$m^2$ area in an 11 x 11 grid.

| Grid | Year | Day | Pellets collected | Total samples | Samples successfully genotyped | New "captures" | Recaptures (within year) |
|---|---|---|---|---|---|---|---|
| C2 | 2020 | 1 | 344 | 61 | 16 | 16 | 0 |
| C2 | 2020 | 2 | 64 | 22 | 10 | 4 | 4 |
| C2 | 2020 | 3 | 67 | 26 | 15 | 9 | 6 |
| C2 | 2020 | Total | 476 | 109 | 41 | 29 | 10 |
| C2 | 2021 | 1 | 18 | 18 | 9 | 8 | 0 |
| C2 | 2021 | 2 | 20 | 20 | 13 | 11 | 2 |
| C2 | 2021 | 3 | 21 | 21 | 20 | 8 | 9 |
| C2 | 2021 | 4 | 34 | 34 | 28 | 15 | 12 |
| C2 | 2021 | 5 | 19 | 19 | 17 | 7 | 9 |
| C2 | 2021 | Total | 112 | 112 | 87 | 49 | 32 |
| E2 | 2021 | 1 | 11 | 11 | 4 | 4 | 0 |
| E2 | 2021 | 2 | 27 | 27 | 16 | 13 | 3 |
| E2 | 2021 | 3 | 14 | 14 | 10 | 5 | 5 |
| E2 | 2021 | 4 | 15 | 15 | 13 | 6 | 6 |
| E2 | 2021 | 5 | 15 | 15 | 15 | 6 | 7 |
| E2 | 2021 | Total | 82 | 82 | 58 | 34 | 21 |
| – | – | Total | 670 | 303 | 188 | 112 | 63 |

In 2020, we identified 29 genotypes on grid "C2", with 20 recaptures (i.e., 20 pellets were genotyped that had previously been identified). In 2021, we identified 48 genotypes on grid "C2", with 59 recaptures. We

identified 34 genotypes on grid “E2”, with 37 recaptures.

*How quickly does genetic material in fecal pellets degrade when exposed to the elements?*

Of the 27 samples exposed to the elements for 0 days, 24 (89%) resolved a full genotype (where ≥5 of 6 loci amplified). All of the samples exposed from 0.5 days to 9 days resolved a full genotype. By day 10 the success rate had dropped to 50% (6 of 12 samples), and by day 60 none of the samples provided a full genotype (0 of 10 samples; **Fig. 3**). The probability of recovering a genotype at a single locus decreased substantially over the sixty days of the trials (**Fig. 3**). The log-odds of recovering a genotype at a single locus were estimated to be 2.17 (95% CI = 1.38–3.14), with a decrease of 0.10 (95% CI = 0.06–0.16) for every additional day the pellet was exposed. The probability of recovering a genotype on the 9th day of exposure was estimated to be 78%.

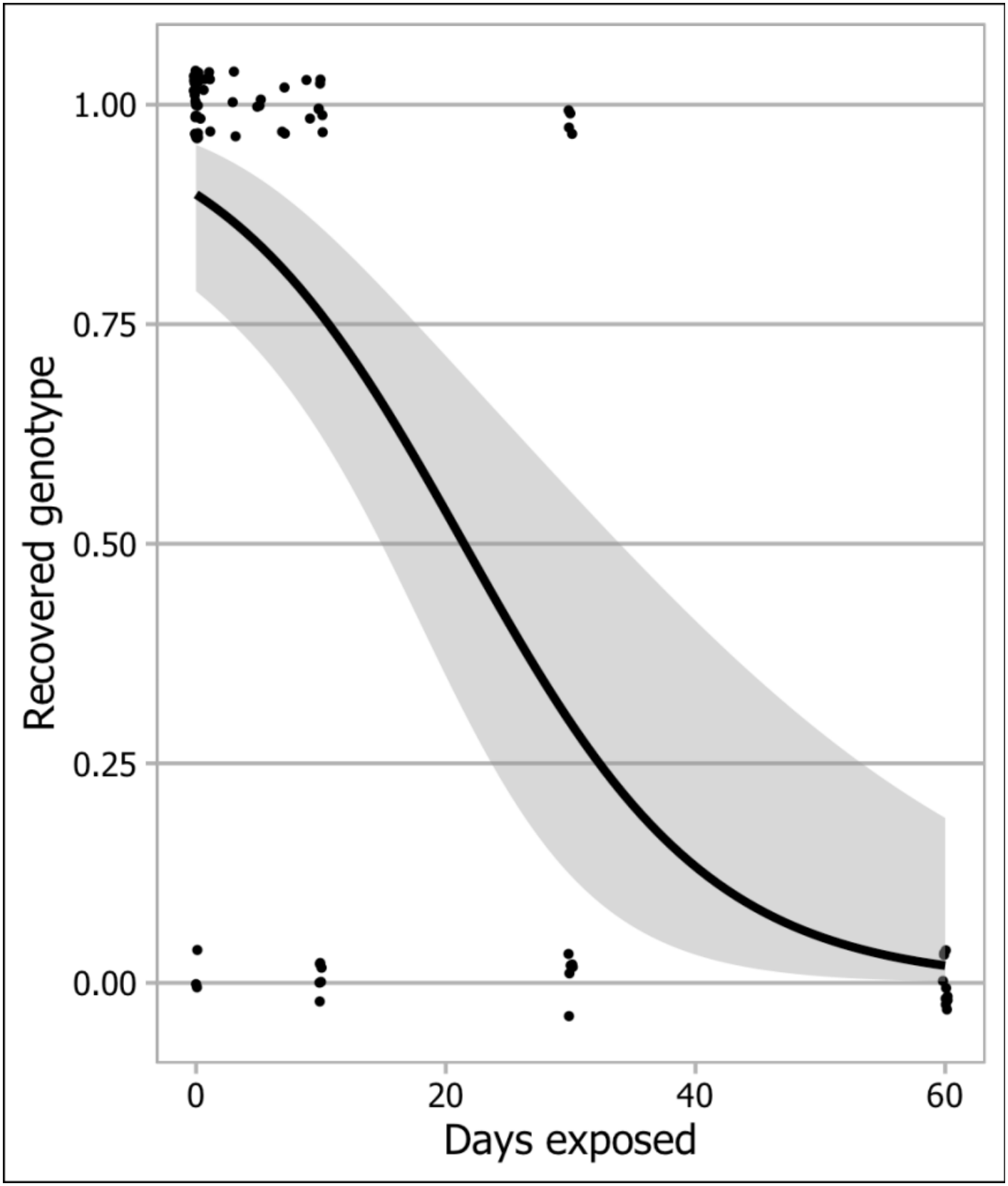


**Figure 3**. Probability of obtaining a genotype at a single locus from giant kangaroo rat fecal pellet in relation to number of days (0, 0.5, 1, 3, 5, 7, 9, 10, 30, 60) exposed to hot, arid conditions *in situ*, 2020–2021. Individual dots jittered for visualization purposes.

*How many pellets are required to resolve to genotype?*

We found that all 14 fresh samples collected from live trapping, both the single pellet and three pellets, recovered a full genotype (i.e., all 11 loci genotyped). We found that day of collection had a greater impact on genotype recovery than number of pellets. In 2020, genotype recovery varied by day ($\chi^2$ = 11.45, P < 0.01), with 17/61 (28%) recovered on day 1, 11/22 (50%) on day 2 and 17/26 (65%) on day 3. We found a similar pattern of differing success on each day of the trial ($\chi^2$ = 29.04, P < 0.001), 16/29 (45%) on day 1, 29/47 (62%) on day 2, 30/35 (86%) on day 3, 41/49 (84%) on day 4, and 32/34 (94%) on day 5. In 2020, success also differed by pellet number ($\chi^2$ = 8.26, P = 0.02): 10/16 (63%) of the single pellet samples collected from the ground recovered a full genotype; 6/14 (43%) of the two pellet samples were successful; and 29/79 (37%) of the three pellet samples were successful.

*How frequently do multiple individuals overlap to contaminate a single sample?*

In 2020, of the 35 stakes that had at least one genotype resolved from fecal pellets, seven had more than one kangaroo rat leaving a fecal pellet. Four of the 45 pooled samples collected on the ground had mixed genotypes. In addition, three different individuals deposited fecal pellets at one stake (“B5”), two individuals on the first night and a new individual on the second night; and two individuals at a different stake (“J7”), one on the second night and one on the third night. In 2021, of the 82 stakes that had a genotyped fecal pellet, seven had more than one kangaroo rat leaving a fecal pellet; in all but one case, they were deposited on different nights.

*How do population estimates from noninvasive genetic sampling compare to live mark-recapture estimates?*

In 2020, we estimated 34 giant kangaroo rats (95% CI = 26–59) from live trapping and 45 individuals (95% CI = 35–75) from the fecal pellet collection on “C2” (**Fig. 4**). In 2021, we estimated 38 individuals (95% CI = 35–48) from live trapping on “C2” and 64 (95% CI = 56–81) from the fecal pellets. We estimated 51 individuals (95% CI = 45–67) from live trapping on “E2” and 43 individuals (95% CI = 38–58) from fecal pellets. Population size estimates from the fecal pellets were lower when subset to just the checkerboard grid: we estimated 30 (95% CI = 18–79), 37 (95% CI = 31–54), and 32 (95% CI = 25–53) individuals on “C2” (2020, 2021) and “E2” (2021), respectively.

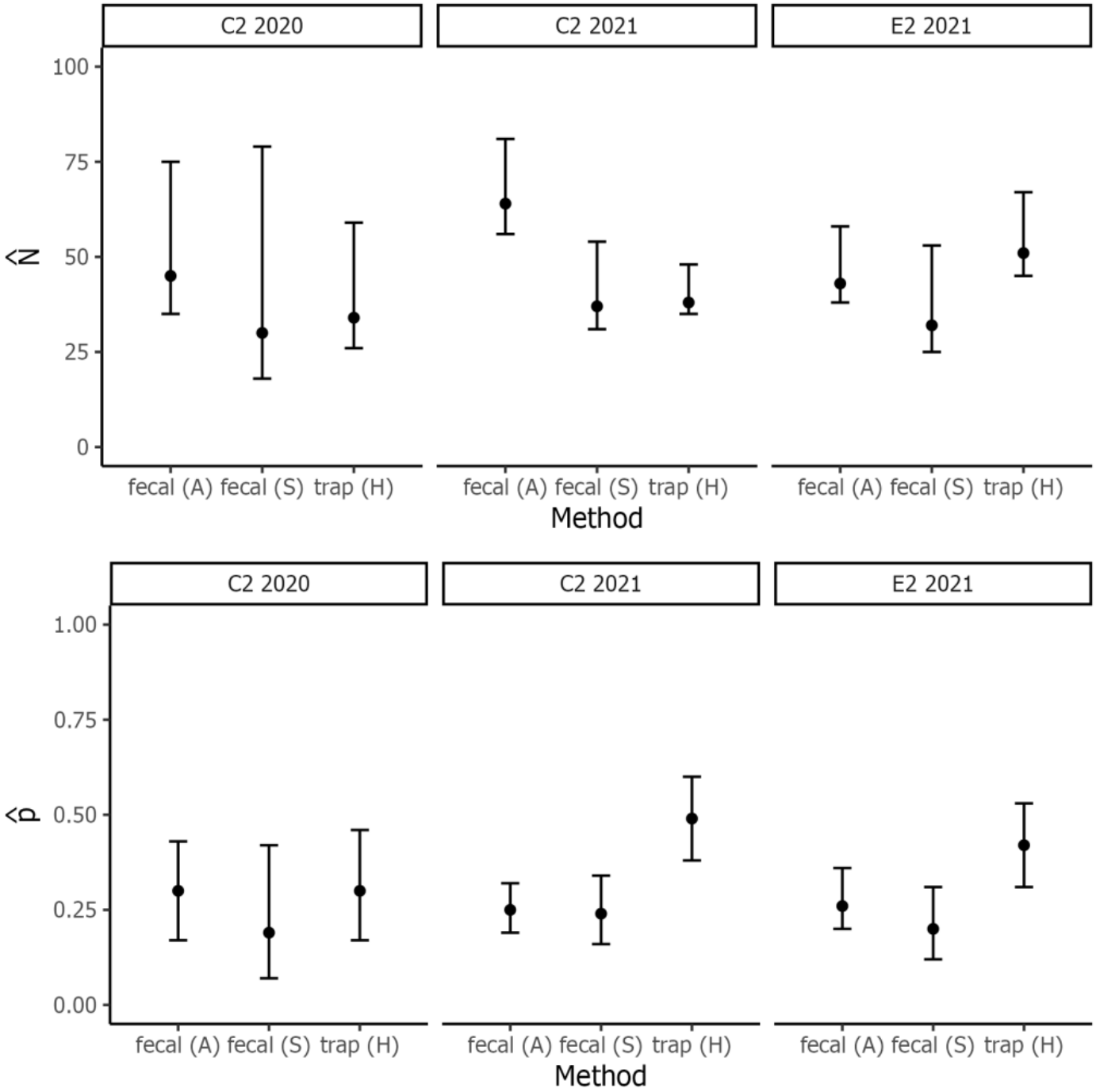


**Figure 4**. Estimated population size (top) and capture probability (bottom) on two grids ("C2" and "E2") in 2020 and 2021 based on two capture-recapture methods with giant kangaroo rats in the Carrizo Plain, noninvasive genetic sampling from fresh fecal pellets and live-trapping. "Fecal (A)" used all of the data available from the collection; "fecal (S)" used a subset from only the points where live traps had been set.

Per-occasion capture probabilities were estimated at 0.30 (95% CI = 0.17–0.43), 0.49 (95% CI = 0.38–0.60), and 0.42 (95% CI = 0.31–0.53) from live trapping on "C2" (2020, 2021) and "E2" (2021) respectively; fecal pellet capture probabilities for the same grids and years were estimated at 0.30 (95% CI = 0.17–0.43), 0.25 (0.19–0.32), and 0.26 (0.20–0.36).

# Discussion

In this study, we addressed a series of questions necessary to develop a robust population monitoring program using noninvasive genetic surveys for small mammals. We found that fecal pellets exposed to hot, arid conditions retained sufficient DNA to identify individuals with a high genotyping success rate for up to nine days, but that there was a precipitous decline after this point. We found that fecal pellets exposed to hot, arid environments degraded within a matter of weeks, and we found that there was sufficient genetic material in a single fresh pellet to reliably identify individual kangaroo rats. Despite the species' territoriality, we also found that pooling fecal pellets from the same location resulted in mixed genotypes comprised of multiple individuals. We found that mark-recapture abundance estimates from noninvasive genetic surveys were statistically similar to estimates from live-trapping efforts.

Noninvasive genetic sampling has been used extensively for population abundance estimation, particularly with carnivores (Owen-Ramos et al. 2022), ungulates (Henk et al. 2022), and primates (Aranjelovic and Vigilant 2018). Overall, 61% (188/303) of our ground samples were successfully genotyped, but results were better in the second year of sampling (75% in 2021) than in the first (38% in 2020). In the first year, we likely over-sampled older pellets, particularly on the first day of sampling. In the second year, observers were instructed to distinguish between new and old fecal pellets. Amplification rates tend to vary considerably across taxa and study systems, but our results are generally on the higher end. For example, in Cabrera voles (*Microtus cabrerae*), 36% (115 of 323) fecal samples resulted in a consensus genotype (Sabino-Marques et al. 2018). By contrast, the amplification rate was much higher in snowshoe hares (*Lepus americanus*), 93.3% (Uhrig 2019). A study of wolves in the northern Rocky Mountains found a consensus genotype in 52% of scat samples (Stenglein et al. 2010). Individual identification from scat samples was 76% in wild tigers in India (Mondol et al. 2009). In Livingstone's fruit bats (*Pteropus livingstonii*), mean genotyping success rate was 7% (Ibouroi et al. 2021). In semi-captive elk, amplification success was 68% (Batter et al. 2022). Even with variation in amplification success between years, our study exhibits similar genotyping success rates to previous efforts and demonstrates the efficacy of this technique for rodents in arid study systems.

Our degradation time trials suggested that a full genotype was >75% probable at nine days of exposure, with a steep decline after that. Although we swept each sample stake clear of any old pellets during each collection day, we likely continued to collect older pellets, thus reducing our genotyping success rate. Such older specimens may have been missed during the sweeping, or they could have blown back onto the sample stakes. On all three grids/years, successful amplification rates increased considerably by day, from a low of 28% on day 1 to a high of 94% by day 5. This latter result was much closer to our estimate of degradation from the time trials for pellets exposed for 0.5 days. The dramatic increase in success rates over the five days was probably due to a combination of the field crew better recognizing fresh pellets, and the older pellets already cleared from the plots (either from sweeping or previous days' collection).

Population size estimates were similar from live capture and fecal pellets in two of the three tests. On "C2" in 2020 and "E2" in 2021, estimates were within a few individuals, with highly overlapping confidence intervals. However, on "C2" in 2021, the population estimate was much higher from fecal pellets compared to live trapping. We identified 49 unique individuals from the fecal pellets, whereas the upper 95% confidence interval from live trapping was 45. It is possible traps were spaced too far apart for a typical giant kangaroo rat home range, or differences in distance to burrows may result in different detection probabilities for each trap. Difference in individual behavior (e.g., trap happy vs. trap shy

individuals) may also have impacted these rates. Estimates of population size from the fecal pellets were lower when subset to just the checkerboard grid of the live-trapping, lending support to this hypothesis. While spatial capture-recapture models could address this limitation of live trapping, giant kangaroo rats that enter a trap almost never use a different trap on subsequent nights or in subsequent sessions, which in this system makes it impossible to use a spatial approach. Efforts to estimate giant kangaroo rat population size using this configuration of live traps (i.e.,20 m apart in a checkerboard) should be considered underestimates of true abundance or density.

## Management Implications

We found that noninvasive genetic sampling using fecal pellets can be an effective, accurate method for estimating population size. Noninvasive genetic sampling can help to avoid many drawbacks of live-trapping, ranging from bureaucratic overhead and animal and researcher welfare to logistics and fundamental problems of individual differences in behavior. Amplification rates were relatively high for giant kangaroo rats, and as costs continue to decline for genetic sampling, this is a promising alternative to estimating population size in this species. However, results from other studies of noninvasive genetic sampling in other small mammal species suggest that amplification rates vary considerably across taxa, system, environmental conditions, and sampling approach. Therefore, species- and system-specific pilot studies should be conducted before developing a population monitoring scheme. Additional studies would also be useful in producing more generalizable expectations.

# Acknowledgments

We thank P. Anderson, R. Vosbigian, B. Sugarman, M. Rose, T. Gaffney, M. Aguilar, E. Francisco, A. Lucero, R. Nolan, and J. Ross for assistance in the field; S. Bird, S. Vanderzwan, A. Lyon, and M. Sadyrova for their help in the lab at UC Davis; J. Hurl and B. Lindquist (Bureau of Land Management) for logistical support (in the midst of a pandemic); and G. Myers, two anonymous reviewers, I. Axsom and P. Anderson for comments on earlier drafts. Funding was provided through a Section 6 grant from U.S. Fish and Wildlife Service, the William and Linda Frost Fund from the Bailey College of Science and Math at Cal Poly SLO, and in-kind support from California Department of Fish and Wildlife and Cal Poly SLO.

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