Spatial and social structure of rewilded laboratory mice

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Highlights

- We describe emergent spatial and social structures of rewilded C57BL/6J (C57) lab mice across replicated 10-day trials in outdoor field enclosures and compare them to wild-derived outbred mice in the same assay.
- Both C57 and outbred males rapidly establish and maintain territories, though the expression of many territorial behaviors are muted in C57 relative to outbred males.
- C57 females explore the field enclosures substantially more than any other group and do not show a bias toward associating with previous cage mates, whereas outbred females show a strong bias in favor of associating with previous cage mates.
- With the exception of C57 females, most mice spent the majority of their recorded time alone.
- We detect significant differences in social structure between the genotypes, highlighting the repeatability of social structure given the same initial starting conditions. The resulting societies formed by C57 mice are less modular and more densely connected than those formed by wild-derived outbred mice.

Keywords: Rewilding, laboratory mice, space use, social structure, territoriality

Abstract

As an essential biomedical model organism, house mice have been studied intensely under laboratory conditions, yet they evolved to survive and reproduce in complex and dynamic natural environments. There has been recent interest in the study of ‘rewilded’ mice in outdoor environments, particularly for understanding the brain and behavior, yet little work has examined lab mouse behavior under free-living conditions. Here, we develop and implement a repeatable field assay to characterize the emergent spatial and social structure of mice. We examine populations of male and female of C57BL/6J (C57) mice over 10 days in large outdoor field enclosures and compare them to populations of recently wild-derived outbred house mice under the same conditions. We observed shared aspects of space use and social structure across all trials but found that C57 societies differed from those emerging from outbred mice across multiple dimensions. Males of both genotypes established and then defended territories, though multiple territorial behaviors are muted in C57 compared to outbred mice. Female C57 mice spent more time associating with other individuals, showed less bias towards interacting with their prior cage mates, and explored more space relative to all other groups. These behavioral differences resulted in C57 mice repeatedly forming more densely connected social networks than outbred wild-derived mice. This work establishes a high-throughput method for examining complex societies of lab mice under semi-natural conditions. Importantly this work demonstrates that C57 mice recapitulate many, but not all, aspects of social structures generated by wild-derived mice in outdoor conditions. Rewilding allows for tractable, replicable, and ecologically realistic approaches to studying mouse behavior and can facilitate the study of the biological basis of higher order social organization.

Introduction

Laboratory house mice are the premier model organism in biomedical research due to their small size, rapid breeding cycle, and the ready deployment of precise experimental manipulations using powerful genetic and neurobiological tools[1–4]. Studying mice in the lab affords tremendous experimental control allowing for the fine-scale dissection of proximate mechanisms across a range of biological fields including genetics, physiology, and neuroscience[2,5,6]. While highly controlled conditions are required for many experiments, there has been a growing recognition that impoverished indoor lab environments limit our ability to understand many complex biological processes[7–9]. Thus, there is a clear imperative to develop approaches that allow for robust studies of the model mammal under more ecologically diverse and realistic conditions. This acknowledgement is especially acute in neuroscience, where a growing number of researchers have highlighted a need to study the brain and behavior in enriched environments that can elicit an animal's full repertoire of natural behaviors[10–16]. Constrained lab environments inherently limit the study of patterns of space use or social behavior that require realistic natural spatial scales relevant to the organism. Even relatively large and enriched lab settings[17–19] fail to capture many of the relevant features of social interactions and social structures inferred by studies of wild mouse populations to be important to mouse natural history, such as territorial social organization and space use[20–24].

An immediate solution is to study the behavior of lab mice in large natural spaces. There is a long history of studies utilizing large enclosures to study the population biology of mice under free-living conditions[25–35]. These studies tend to use feral or wild-derived populations of outbred house mice and find that male mice establish and aggressively defend territories occupied by several females and their offspring. Fully adult males are most often associated with high quality territories, while juveniles and subadults typically aggregate in lower quality spaces within the environment[26,36,37]. Adult females may compete for nest sites but form close associations and may even co-nest with close relatives[38,39].

Yet, whether these same behaviors and structures emerge in societies of well-studied laboratory mice living under comparable ecological conditions remains unknown. Indeed, multiple lines of evidence demonstrate that lab mouse strains commonly used for behavioral research differ from their wild counterparts in aspects of their behavior and physiology likely arising from generations of inbreeding, artificial selection for fecundity and docility, and rearing in chronically impoverished cage environments[2,40–44]. Previous studies of rewilded lab mice in outdoor enclosures[45–49] have not detailed

the social behavior or emergent social structure of these animals. As a result, fundamental features of lab mouse behavior under free-living natural conditions remain poorly understood.

Here we report the space use and social behavior of replicated populations of the common laboratory strain C57BL/6J (C57) in large outdoor field enclosures located in Upstate New York, USA. In addition to developing a robust and high-throughput field assay of mouse spatial and social behavior, we aimed to address three empirical questions with this study. (1) We sought to determine whether male lab mice would establish and defend territories in the field, as opposed to generating societies with a single integrated dominance hierarchy (a common outcome in the lab[19,50–52]). We also compare the territorial behaviors of lab mice and wild-derived outbred mice. (2) We sought to test whether space use and the resulting social relationships that form differ between C57 and outbred females. Female fitness in the wild depends on their abilities to compete for nesting sites[38] and they show spatial and social biases toward other related females[39]. We hypothesized that lab mouse husbandry practices, which regularly lead to isogenic females living and occasionally breeding at very high density[1], may have led to changes in the magnitude of social biases towards familiar females. (3) Finally, we sought to describe the social networks that emerged from these individual behaviors, and how differences in behavior between C57 and outbred animals scaled up to shape larger social structures. We hypothesized that, despite the myriad complexities and idiosyncrasies of individual decisions that contribute to larger societies, repeated studies of societies that differed in a salient manipulation—in this case the genotype of the individuals in the society—would result in consistently different outcomes across treatments. If this hypothesis is correct, populations with similar initial ecological and demographic conditions will reliably generate similar social structures, suggesting that the biological basis of social organization is amenable to study.

Results

Field approach to studying lab mouse space use and social structure

Field studies of wild populations provide a powerful means to link aspects of organismal biology to selection, but are typically hampered by a lack of replication[53,54]. Enclosure studies done over short, but biologically relevant, time periods provide an opportunity to observe replicate populations across multiple trials. A fundamental feature of enclosure studies, as compared to wild populations, is that the experimenters must choose among a range of possible resource distributions, population densities, sex ratios, etc. as the starting conditions of the trials. House mice naturally live under a wide range of

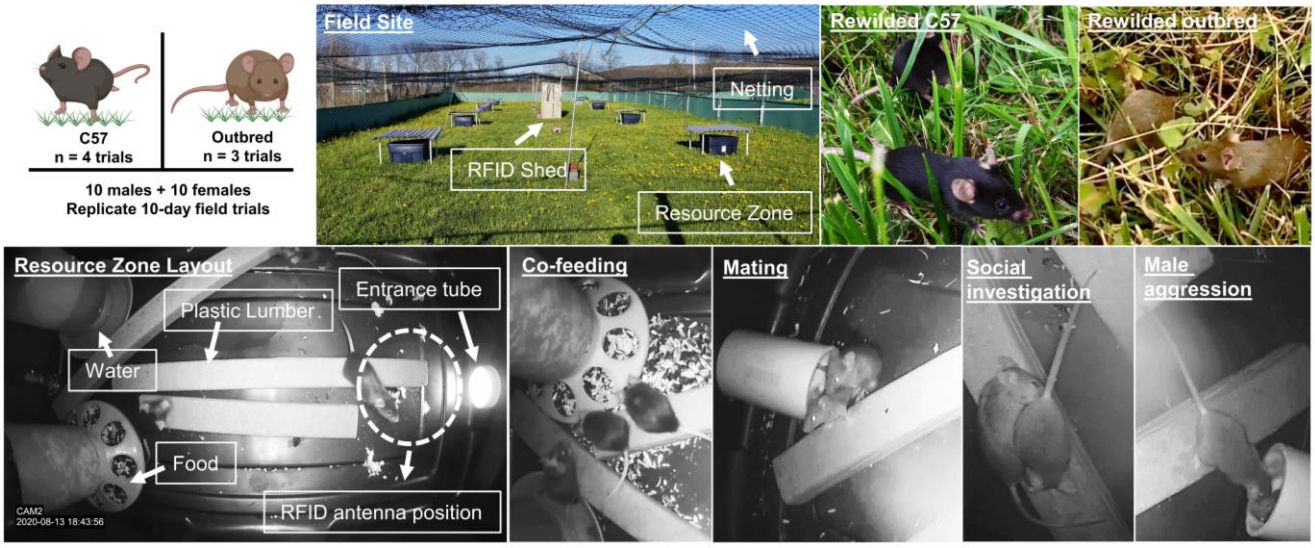

Figure 1: Experimental design for replicate populations of C57 and outbred mice in field enclosures Photos demonstrate the layout of the field enclosures and the eight resource zones arranged in a 2x4 grid pattern. Resource zones had a single entrance tube and food and water towers provisioned *ad libitum.* We observed a variety of behaviors in the resource zones including co-feeding between females, mating and courtship, social investigation, and male-directed aggression towards intruders. Top left image created with BioRender.

resource distributions and densities across non-commensal and commensal settings[26,31,37], meaning that there exists a large set of naturalistic conditions rather than a single most appropriate condition for studying mice. While studying social organization across a range of socio-ecological starting conditions will be informative, for this study we chose a single common set of initial resource distribution, density, and sex ratio conditions for all trials. This experimental design allowed us to assess the consistency of social behaviors and structures formed within the enclosures across replicates and to isolate the effect of host genetic background on these outcomes. Moreover, the approach demonstrated here could be used with a variety of demographic compositions or resource distributions providing a flexible paradigm to study mouse behavior under field conditions.

For our experiments, we chose the following conditions: Over a three-month period (June 2020 - August 2020), we examined the emergent social organization generated in enclosures stocked with 10 female and 10 male house mice (*Mus musculus domesticus*) from the well-studied lab mouse strain C57 (n = 4 trials) and outbred wild-derived house mice (n = 3 trials). Rather than merely qualitatively comparing our data to published data on mouse behavior in natural or semi-natural settings[22,28,36,55,56], we conducted identical, simultaneous trials using outbred wild-derived house mice to allow for a direct quantitative comparison of data collected using the same methods and the same physical environmental

conditions. We note that while lab mouse ancestry includes three house mouse subspecies, the C57 genome is >90% *Mus musculus domesticus*[57]. Therefore, as a 'control' wild mouse population, we used outbred mice generated from multiple *Mus musculus domesticus* lines derived from wild house mice caught in upstate New York. Thus, the comparison group of mice were born and raised in the lab for multiple generations but have an outbred wild-derived genetic background. The population density in our enclosure was 0.034 mice/m^2, which falls within the range of typical population densities reported for wild mice[58] (~0.0011 – 0.11 mice/m^2).

Our outdoor field enclosures are approximately 9,000 times larger than the area of a standard laboratory mouse cage (~570m^2; **Fig. S1B**). Each field enclosure contained eight weather-protected, defendable resource zones (made from 32-gallon rubber storage tubs), which were evenly distributed in a 2x4 grid (**Fig. 1**; **Fig. S1A-B**). This choice meant that there was a slight excess of mice of each sex relative to resource zones. We supplied all resource zones with food and water accessible by the mice *ad libitum.*

We monitored the zones continuously over the trial period via a radio frequency identification (RFID) antenna placed beneath the sole entrance into the zone (**Fig. 1**; **Fig. S1C**). Since the RFID antenna detected tags both above and below the horizontal plane of the antenna and mice moved underneath the resource zones, we inferred RFID reads to indicate that mice were within a space inclusive of the inside and underside of the storage tubs. To initiate each trial, we placed mice implanted with passive RFID tags into one of the eight resource zones with their same-sex sibling cage mates in the evening shortly before sunset, meaning that all individuals started the trials at a resource zone with one or more familiar social partners (see Supplementary Material for details on cage mate and relatedness information).

We allowed trials to proceed for 10 days. This trial length was chosen because it allowed us to avoid females giving birth in the enclosures and made it feasible to conduct multiple replicated trials in the same enclosures over the course of a single summer. House mouse social structure has been reported to vary seasonally and with shifts in demographic parameters in natural populations[20,56,59], so we note that different conditions or studies conducted at other times of year may yield different results. The experiment reported here focuses on behavior during the initial formation and establishment of social structures within replicated mouse populations in our enclosures while controlling for seasonal and demographic variation. Over the course of 10 days, mice explored the enclosures and resource zones and engaged in a variety of social interactions with conspecifics including courtship, mating, co-nesting, and

fighting (**Fig. 1**; **Video S1**). As our goals focus on testing hypotheses regarding patterns of mouse space use and social structure, we focus our analyses here on the large RFID dataset. We obtained high density sampling of mouse RFID reads for all trials (1,307,712 ± 135,646 reads per trial; mean ± SE) and a mean of 6771 ± 275 RFID reads per mouse per day (see Supplementary Material). Mice were able to quickly traverse the distance between the zones despite the ground vegetation (minimum inter-zone travel time = 10 seconds; **Fig. S1D**).

To convert instantaneous RFID reads into estimates of how long mice spent at the resource zones, we grouped RFID reads into discreet resource zone visits with estimated durations (**Fig. S1E-F**; see Methods for details). As expected, the total number of visits to a zone strongly predicted the total estimated duration of time spent at a zone (Spearman's correlation, $R > 0.84$ for all genotype and sex combinations; **Fig. S1G**). Using this approach, we estimated individual mouse location for a total of 5653 hours across all trials (mean = 808 ± 51 hours/trial; see Supplementary Material). On average, we inferred that individual mice spent 4.2 ± 0.1 hours/day at resource zones (range: 1.1 seconds – 18.7 hours). Using these data, we describe patterns of space use and the social structure that emerged during the trials.

Territory establishment is slower in C57 compared to wild-derived outbred males

Territorial behavior is reported from wild mouse populations and field enclosure studies at moderate density or lab environments with extremely high physical complexity[28,36,60–62]. In mice, territorial males exclude (or at least attempt to exclude) other males from the spaces that they control. The ability to compete for and maintain a territory is a key driver of male fitness in freely mating mouse populations[35]. The widely used resident-intruder assay in studies of lab mouse social behavior is predicated on the fact that male mice tend to defend spaces[63]. An alternative social structure, where groups of male mice form a social hierarchy has been observed regularly in the lab and in some natural settings where there is low environmental complexity such that individual dominant mice can readily patrol most of the area[19,50,51,64]. Male mouse social hierarchies can vary in the extent of despotism or egalitarianism[19], though are generally characterized by a dominant alpha mouse who is aggressive to all others and has broad access to space, followed by a linear hierarchy of subordinates[52]. Commensal mouse populations may show a mixture of these social forms, with hierarchies formed among the males residing within a single territory or deme but are nevertheless characterized by restricted movement of mice between territorial spaces.

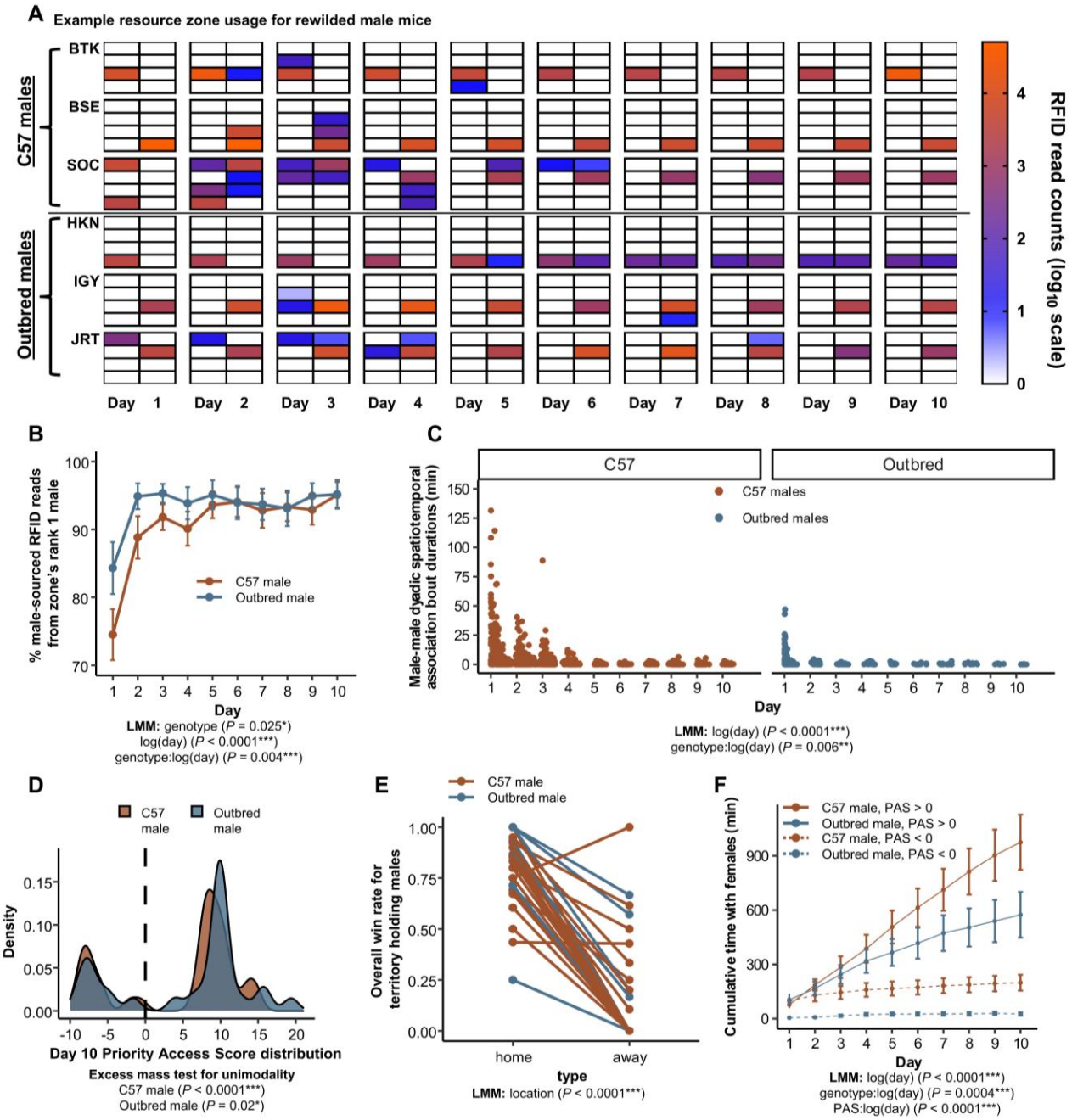

Figure 2: Both C57 and outbred male mice establish territories rather than dominance hierarchies
(A) Males generally visited only one or two zones, and no males visited all zones, as shown by representative space use data from 3 C57 and 3 outbred males. Each 2x4 grid shows the eight resource zones on each of the 10 days of a trial, with redder colors indicating a high concentration of RFID reads. **(B)** Outbred males form territories more quickly than C57 males. Here the y-axis represents the percent male-sourced RFID reads per zone from the focal zone's most present male, with values of 100% indicating complete monopolization. **(C)** Male-male dyadic spatiotemporal association bout events were shorter, less frequent, and deteriorated more quickly in outbred mice compared to C57 mice. **(D)** To quantify territoriality, we calculated a Priority Access Score, a cumulative metric of resource zone access across the 10 days of the trial. For both outbred and C57 males, this territory metric was deviated from unimodality, showing a group of males that had consistent access

to resource zones (territory holders) and those without (floaters). Here scores near or greater than +10 indicate a male that controlled a resource zone for the duration of the trial. Negative scores are indicative of males that failed to capture a territory. **(E)** Consistent with territoriality, but not a dominance hierarchy, territory-holding males were much more likely to win a spatial dispute with another male, defined as displacing the other male, when the dispute occurred in their home territory as opposed to in a different male's territory. **(F)** Territorial control (as categorized by having a Day 10 PAS > 0) conferred benefits in the form of increased access to females. This benefit of territoriality was stronger among outbred as compared to C57 males (notice that outbred males without a territory essentially never spend any time with females, dashed blue line).

We sought to determine whether males in our enclosures formed territories or dominance hierarchies. If males form territories in our enclosures, a subset of the males in each trial would each monopolize one or more resource zones to the relative exclusion of others. What's more, no male would regularly access all resource zones. In contrast, if males establish a dominance hierarchy across the enclosure, the top-ranking male or males are expected to have relatively free reign and regularly access all or nearly all resource zones.

Consistent with previous field enclosure studies[36], we find evidence that males of both genotypes established and defended territories, though the intensity of territorial behaviors was muted in C57 relative to the outbred mice. Three related, but distinct analyses support this conclusion.

First, males appear to establish monopolized spaces and rarely visit resource zones that they do not control (**Fig 2A; Fig. S2A**). By the third day in the enclosures, 93% ± 1 of all male-sourced RFID reads within each resource zone belonged to a single male, with zones in outbred trials becoming monopolized by males more rapidly than in C57 trials over the course of the trial (t_{500} = -2.93, P = 0.004; **Fig. 2B**). What's more, no individual male consistently accessed all or nearly all resource zones (range of mean daily zones visited = 1.0-3.9, median = 1.6; in contrast to C57 females**,** see **Fig. 3B** below). Instead, males spent the vast majority of their time within their single most visited resource zone, with outbred males developing this site fidelity more rapidly than C57 males (t_{67} = 2.31, P = 0.02; **Fig. S2B**). Thus, male space use was consistent with territory defense, and inconsistent with a single integrated dominance hierarchy.

Second, males spend extremely little time in spatiotemporal overlap with other males. In all trials, males were placed into the enclosure within a resource zone along with their cage mate (brother). Males therefore overlapped in space at the start of the trials, but episodes of male spatiotemporal overlap rapidly decreased in both frequency (t_{58} = -9.47, P < 0.0001, **Fig. S2C**) and duration (t_{2116} = -13.15, P < 0.0001, **Fig. 2C**). The collapse of previously existing cage mate relationships was remarkably rapid in outbred males: on average, at least half of all the estimated dyadic spatiotemporal overlap among

outbred males elapsed in the first 54 minutes of the trial (range = 32 – 93 minutes). The same phenomenon occurred more slowly in C57 males with at least half of all estimated dyadic spatiotemporal overlap occurring in the first 590 minutes of the trial on average (P = 0.017; range = 210 – 1501 minutes). Male-male spatial overlaps shortened in duration over the course of the trial more quickly in outbred males as compared to C57 males (t_{2116} = -2.77, P = 0.006, **Fig. 2C**) and were less frequent overall (t_{27} = -4.14, P = 0.0003, **Fig. S2C**), suggesting that the intensity of male-male competition in outbred mice may be greater than that of C57 mice. Finally, we found that males spent much less time in spatiotemporal overlap on days 5 – 10 of the trials than we would expect if territorial and intruder male behavior were independent. Strikingly, the amount of temporal overlap between a dominant territorial male in a given zone and other males that visited that same zone was 86% less than expected if each males' space use was independent of each other (range across trials = 74% - 97% less; t_6 = -58, P < 0.0001). This pattern could be explained either by (a) territorial males quickly expelling intruders when they co-occur or (b) non-territorial males biasing their visitation behavior to times that they expect the territorial male to be absent.

To characterize the variation in males' territorial behavior and resource access, we calculated a daily resource zone Priority Access Score (PAS; see Methods for full description). We calculated this daily metric for each mouse based on the duration of time we estimated a mouse spent at a zone relative to all other same-sex individuals. Briefly, mice gained 1 point for each resource zone they fully monopolized on a given day or a fraction of a point for partial monopoly. Mice that failed to monopolize any zone (<50%) were given a -1 penalty. Thus, for the 10-day trials reported here, strongly positive final scores (near +10) indicate an individual consistently excluded others of the same sex from a single resource zone on each day of the trial while strongly negative scores (near -10) indicate an individual was consistently excluded from most spaces. High scores (>>10) indicate individuals who monopolized more than 1 zone over the course of the ten-day trial. Scores closer to zero indicate individuals that share spaces to some extent with other individuals of the same sex. Consistent with the hypothesized presence of territorial and non-territorial ('floater') males we find a bimodal distribution of males with high and low PAS values (excess mass test for unimodality: P < 0.02; **Fig. 2D; Fig. S2D;** in contrast, females of both genotypes do not strongly monopolize resource zones at this density, see **Fig. S3B-C**).

The third and ultimate indicator of territory formation rather than a single dominance hierarchy would be for contest outcomes to be predicated on ownership. In other words, for territorial males to win competitive interactions in their territory and lose in other males' territories such that encounter

outcomes depend on location. In contrast, under a social dominance hierarchy, a given male should be equally likely to win or lose depending on their rank without respect to location. To assess this prediction, we used the RFID data set to investigate male-male dyadic interactions within territories by identifying when the territory-holding male spatiotemporally overlapped with an intruder. For each such inferred overlap, we identified which animal was the first to leave the interaction (i.e., was displaced and lost acute resource access) and assigned that male as the 'loser' of the interaction event. We assigned the remaining male (for whom RFID reads were still detected) as the 'winner'. We identified 1380 such events which, as expected, overwhelmingly involved territory holders (n = 1290 events, 93.5% across all trials; mean = 25.6, median = 14, 95% C.I. [15.8, 35.2] per territory holding male). To compare win and loss rates for the same individuals, we examined 32 territorial males that were observed to engage in both 'home' and 'away' displacement event (n = 23 C57, 9 outbred; many territorial males were not observed in displacement events 'away', i.e., zones they did not have the majority of RFID reads). We find that overall win rates of territory holders at home (0.82 ± 0.03) are dramatically higher than win rates away (0.18 ± 0.05; t_{31} = 12.19, P < 0.0001; **Fig. 2E**), with only one male showing a lower win rate at home than away. The one male that had an excess win rate away was only observed to participate in single away displacement event, which he won. More than half of the males (59%, 19/32) lost all their contests when away from their territories. Winning away was rare after the first day of the trial, when boundaries were still in flux; 72% (50/69) of displacement events won away occurred on the first day of the trial. The universally high win rates of territorial males at home suggests that our method is not misidentifying subordinate or squatting floater males as territory holders (otherwise, we would expect to see some animals with win rates near 0 at home). Together, these results support the territory hypothesis and reject a hypothesis where male mice in the enclosures formed a single integrated dominance hierarchy.

Territorial control was associated with increased access to females. Males with positive (>0) final PAS values spent more total time with females than males with negative (<0) final PAS values over the course of the trial (t_{661} = -3.6, P = 0.0004; **Fig. 2F**). The effect of territoriality on males' access to females was stronger among outbred males. Among outbred males, those with low PAS values essentially never spent any time with females (range of total time spent with females = 0.73 – 74.5 minutes), even on the first day of the experiment. In contrast, although C57 males with low PAS values spend dramatically less time with females over the course of the experiment as compared to high PAS males, they spend comparable amounts of time with females during the first day of the experiment,

suggesting that the competitive exclusion of males from access to females was slower to develop in C57 mice.

Establishment of non-overlapping territories led to similar patterns of space use in both C57 and outbred males. Examples of males' spatial behavior are shown in spatial heatmaps in **Fig. 2A** highlighting the spatial separation and fidelity of males in a single trial (see **Fig. S2A** for full example trials). The males in each trial that were not able to capture a resource zone (those with Priority Access Scores less than 0) appeared to adopt an alternative 'floater' strategy in which they briefly visited several zones each day. These floater males, who were not tied to a specific space that they needed to defend, tended to visit more zones over time on average as compared to the territorial males (t_{65} = 2.61, P = 0.01; **Fig. S2F**).

Distinct patterns of space use and social associations in C57 females

Studies of free-living house mice document competition among females for nesting space. Females tend to tolerate their relatives while avoiding or even showing aggression towards unrelated females[39,65,66]. At the same time, male territorial structure may shape female behavior as novel males can lead to pregnancy termination through the Bruce effect[67,68] and novel males represent an infanticide threat[67–70]. Competition for nesting space and avoidance of non-sire males is likely to be most acute among breeding females[71,72]. Though our trials were too short for females to give birth, they allow us to examine how females use space when first introduced into a large novel social environment and assess the extent to which their space-use behaviors are influenced by their social environment.

C57 females exhibited substantial differences in space and movement patterns across several measures compared to outbred females as well as both male genotypes (**Fig. 3A; Fig. S3A; Fig. 2A; Fig. S2A**). Unlike outbred females and males of both genotypes, C57 female space use was not limited to a few neighboring resource zones, but instead was widespread across the enclosure space. Across sexes and genotypes, mice visited an average of 2.32 ± 0.11 resource zones per day over the course of the trial, though patterns of zone visits varied over time and among individuals. On average, the number of resource zones that animals visited each day increased as the trials progressed (t_{133} = 13.3, P < 0.0001), but this increase was driven entirely by the behavior of C57 females (P = 0.52 for non-C57 females; **Fig. 3B**). Although all mice explored an equivalently low number of resource zones during the first several days in the enclosure, by the fourth day C57 females had significantly increased exploration

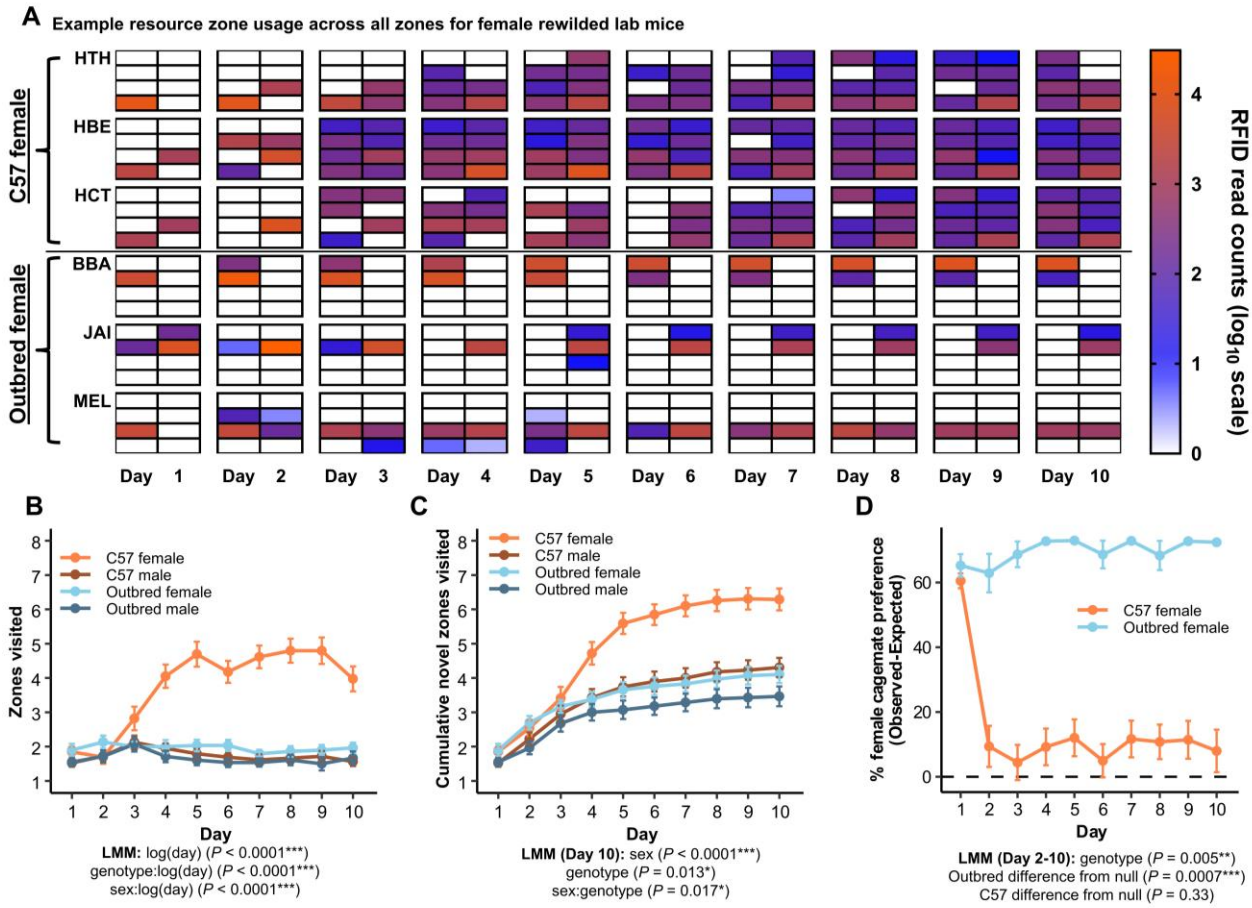

Figure 3: C57 females show distinct patterns of space use and social associations
(A) C57 females explore more of the available resource zone spaces than C57 males and outbred males and females, as shown by three representative individuals from each strain. Each 2x4 grid shows the eight resource zones on each of the 10 days of a trial, with redder colors indicating a high frequency of RFID reads. **(B)** All sex and strain combinations visit similar numbers of zones (unique per day) on the first several days of the trial, but C57 females increase the numbers of zones visited relative to any other sex or strain combination as the trial continues. **(C)** C57 females visit more cumulative resource zones over the course of the trial period compared to all other sex and strain combinations. **(D)** Outbred females show a strong and sustained preference for their cage mates while C57 female preferences for cage mates quickly fall to null expectation levels (as indicated by the dashed line). Note that mice were placed in resource zones with their cage mates on the first day of the trial.

of the available zones compared to all other groups ($P < 0.05$ for daily LMM contrast estimates for Day 4 – 10), which did not differ in their extent of space use.

In addition to visiting more zones on average per day, C57 females visited a greater proportion of all possible zones over the course of the trial (**Fig. 3C**). By the final day of the trial C57 females had visited 6.3 ± 0.4 of the 8 available zones, which is more than C57 males (4.3 ± 0.4; $t_{125} = 5.5$, $P <$ 0.0001), outbred females (4.1 ± 0.5; $t_{7.4} = 3.3$, $P = 0.045$), and outbred males (3.5 ± 0.5; $t_{7.6} = 4.3$, $P =$

0.01). Many more C57 females (42%, 16/38) visited all 8 resource zones as compared to C57 males (8%, 3/39), outbred females (3%, 1/29), and outbred males (4%, 1/28) (generalized LMM: $P < 0.05$ for all comparisons). Finally, C57 females spent less time in their most occupied zone compared to outbred females ($t_{18} = 2.3$, $P = 0.04$; **Fig. S3E**).

Female house mice exhibit social preferences towards familiar same-sex conspecifics under free-living conditions[39,66], but the degree to which this happens in lab mice is unclear. Our results suggest that C57 females are more tolerant towards females in general and less biased towards familiar social partners. Overall, C57 mice engage in longer female-female spatiotemporal association bouts compared to outbred mice over the course of the trial ($t_{11910} = -2.8$, $P = 0.023$; **Fig. S3F).** At the same time, the female preference for spending time with cage mates on days 2-10 of the trials was much greater in outbred females than in C57 females ($t_5 = 4.8$, $P = 0.005$). Outbred females ($t_5 = 7.2$, $P = 0.0007$), but not C57 females ($t_5 = 1.1$, $P = 0.33$), differed significantly from the null value based on the prevalence of cage mates within a trial (**Fig. 3D**). Indeed, outbred females spent nearly all their female-female social time with cage mates after day 2, whereas C57's tended to spend less than half of their time with cage mates (**Fig. S3G**). These results suggest that inbreeding and/or lab mouse colony rearing practices may have a profound effect on both female space use and social behavior.

Repeatable differences in social structure between C57 and outbred mouse societies

Analyses of the behavior of each sex show generally similar patterns of space use for males but strongly divergent patterns for females. We next sought to understand how behavioral differences measured for individuals scaled up to larger patterns of social association and the emergent structure of societies.

We first examined how mice overlapped in space and time to determine to what extent individuals associated, as well as the range of spatiotemporal group compositions that arose. For each trial we estimated the time spent in group compositions of differing numbers of males and females (**Fig. 4A**). For this analysis, we considered each mouse's time separately such that a pair of mice in a zone together for 60 minutes is recorded as two mouse hours. In each trial, mice were alone for most of the time that they were recorded at resource zones (range 50-64% for C57; 70-82% for outbred mice, solitary mouse time per trial; $P = 0.029$ based on the probability of all four C57 trials having the lowest values; **Fig. 4A)**, but the proportion of time that individuals spent alone was strongly predicted by sex and genotype. On average, males spent a greater proportion of recorded time at resource zones alone

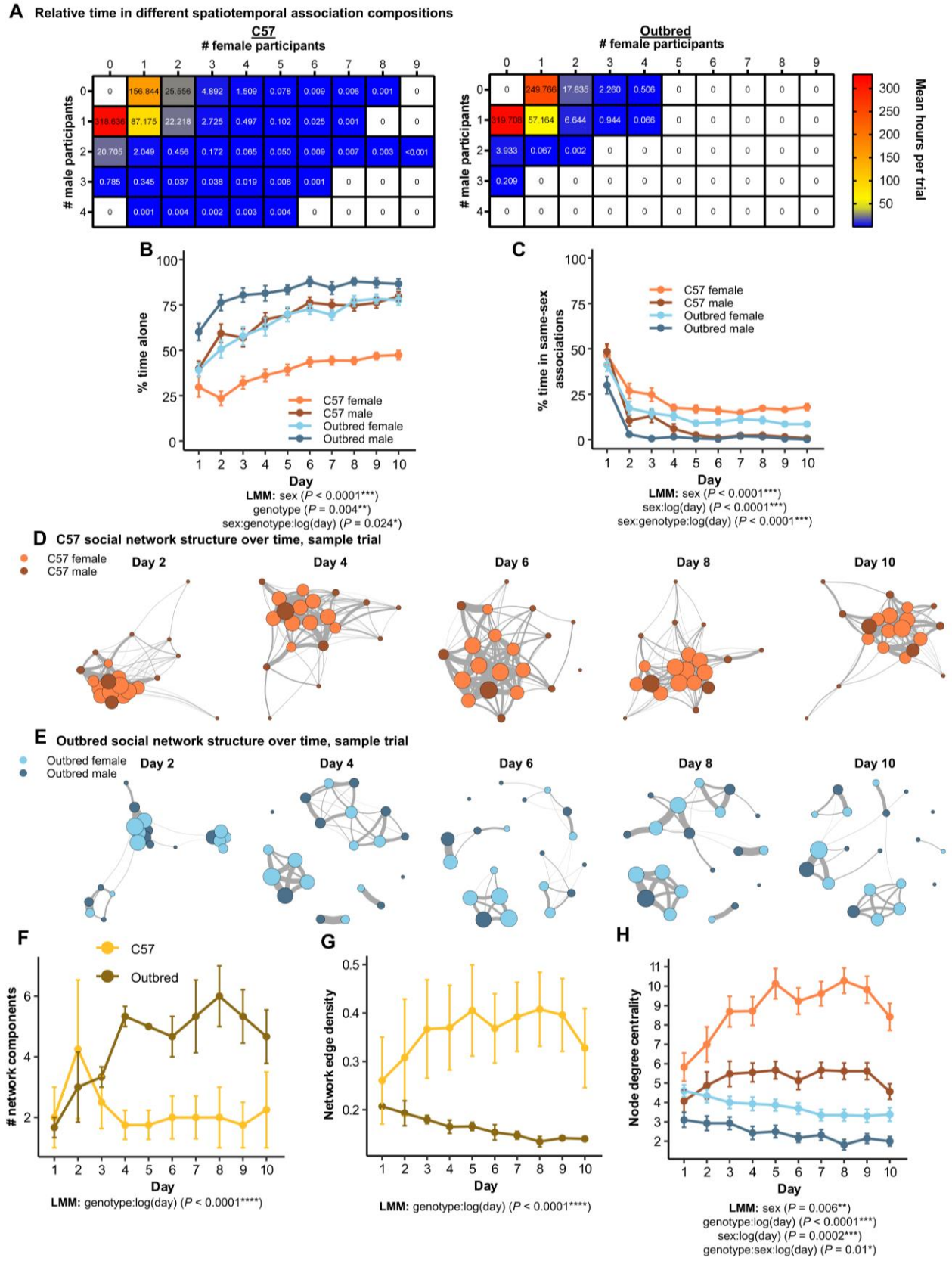
A Relative time in different spatiotemporal association compositions
C57
female participants
male participants
Outbred
female participants
male participants
Mean hours per trial
B
% time alone
C57 female
C57 male
Outbred female
Outbred male
Day
LMM: sex (P < 0.0001***)
genotype (P = 0.004**)
sex:genotype:log(day) (P = 0.024*)
C
% time in same-sex associations
C57 female
C57 male
Outbred female
Outbred male
Day
LMM: sex (P < 0.0001***)
sex:log(day) (P < 0.0001***)
sex:genotype:log(day) (P < 0.0001***)
D C57 social network structure over time, sample trial
C57 female
C57 male
Day 2
Day 4
Day 6
Day 8
Day 10
E Outbred social network structure over time, sample trial
Outbred female
Outbred male
Day 2
Day 4
Day 6
Day 8
Day 10
F
C57
Outbred
network components
Day
LMM: genotype:log(day) (P < 0.0001****)
G
Network edge density
Day
LMM: genotype:log(day) (P < 0.0001****)
H
Node degree centrality
Day
LMM: sex (P = 0.006**)
genotype:log(day) (P < 0.0001***)
sex:log(day) (P = 0.0002***)
genotype:sex:log(day) (P = 0.01*)

Figure 4: C57 social structures are more interconnected than in outbred mouse societies
(A) C57 mice spend less time alone and have larger numbers of social participants in association events as compared to outbred mice. The contour plot shows the average duration of observed time spent in different male and female group compositions across trials for C57 (left) and outbred (right) mice. The numbers reported here are the average number of hours estimated per trial. **(B)** C57 females spend less than half of their total observed time alone throughout the trial, less than any other group. **(C)** All strain and sex combinations decrease the time spent in same-sex groups over the course of the trial, with males of both strains spending minimal amounts of their observation time in same-sex interactions. **(D-E)** Daily social networks from an example C57 trial **(D)** demonstrates a typical pattern of persistently high female interconnectivity while an example outbred trial **(E)** demonstrates increasing network modularity over time. The size of connections between nodes represents the edge weight while the size of nodes reflects the node edge strength, or the sum of all edge weights for a single node. **(F)** Number of network components increases in outbred, but not C57, social networks over time. **(G)** Network edge density increases in C57, but not outbred, social networks over time. **(H)** C57 females have consistently higher measures of node degree centrality, indicating that they are consistently meeting large numbers of social partners on each day of the trial, relative to other sex and strain combinations.

than females (t_{1339} = 5.4, P < 0.0001; **Fig. 4B**). Overall, outbred males increased their time alone over the course of the trial (t_{1339} = -3.1, P = 0.002; **Fig. 4B**) and were especially likely to spend time alone compared to all other groups; all of them (29/29) spent more than 50% of their total recorded time alone. In comparison 83% of outbred females (25/30), 78% of C57 males (31/40), and only 18% of C57 females (7/40) spent most of their recorded time alone. Given the interest in the biology of social isolation in mice[73–77], it is notable that when given the opportunity to freely interact, many mice spent a significant portion of their observed time alone over the course of their trial.

Though individuals spend a large portion of their time at the resource zones by themselves, we estimated more than 2000 mouse hours of spatiotemporal associations across the seven trials, defined as time with two or more mice at the same zone simultaneously (**Fig. S1F**). Dyadic interactions accounted for >50% of estimated association time in both genotypes (62-80% in C57, 78-86% in outbred), though larger aggregations of mice were detected in all trials (**Fig. 4A**). On average, females spent a greater portion of their recorded time in same-sex associations than males over the course of the trial (t_{1339} = -6.5, P < 0.0001; **Fig. S4A**). Most mice (77%, n = 103/134) spent >50% of their recorded association time in opposite-sex groups, with males increasing this metric over the course of the trial relative to females (t_{1165} = 6.6, P < 0.0001; **Fig. S4B**).

To investigate the emergent group structure of both genotypes, we analyzed the total and daily social networks formed for each trial. Overall, C57 mice formed more connected networks than outbred mice, a difference which was largely driven by high levels of C57 female sociability (**Fig. 4D-E**).

Outbred networks increased in the number of graph components – the portions of the network disconnected from each other – over time (t_{61} = 4.86, P < 0.0001; **Fig. 4F**), echoing the demic structure reported for many wild mouse populations[23,78,79]. In contrast, C57 networks became more connected over the course of the trials. Over time, the network edge density – a measure of the proportion of edges actually observed out of all possible edges in the network – increased in C57 social networks, but not in outbred networks (t_{61} = -5.4, P < 0.0001; **Fig. 4G**). Notably, the two genotypes show completely non-overlapping distributions for network edge density from days 4-10 (P = 0.029 each day). We note here that these difference between genotypes reported in this section are based on whole network level metrics indicating that the basic overall structure of social organization differs between the genotypes.

Females of both genotypes had high degree centrality measures compared to their respective males, indicating females encountered more unique social partners on each day of the trial. There was a significant three-way interaction between sex, genotype, and time, such that C57 females rapidly increased their network centrality measures compared to all other sex and genotype combinations (t_{134} = 2.6, P = 0.01; **Fig. 4H**). Thus, many of the differences we see in social networks between the genotypes is driven by the propensity of C57 females to co-occur with many distinct individuals. By the final day of the trial, C57 mice had overlapped with many more of the available social partners present in the enclosures as compared to outbred mice ($t_{5.48}$ = -3.9, P = 0.0098; **Fig. S4C**), who failed, on average, to ever meet more than 50% of the potential social partners. Intriguingly, females of both genotypes showed high levels of vertex page rank scores, indicating that females of both strains maintain higher levels of connectedness over time than their respective males (t_{1343} = 3.2, P = 0.001; **Fig. S4D**).

Discussion

Our replicated field experiments demonstrate that C57 lab mice broadly recapitulate many of the behaviors of wild-derived mice in free living conditions, including clear evidence of male territories, but have different emergent social structures. This difference is largely due to C57 females being more exploratory and showing less biased patterns of social association. The organization of mammal societies is influenced by ecological[80–82], demographic[83–86], and phylogenetic factors[87–89], each of which were controlled for in our trials. Thus, these data show that genotype can have a strong effect on social structures in mammals[90,91]. These data also highlight the flexibility of mouse social behaviors across diverse ecological and demographic conditions. For example, in contrast to lab studies at high density, which identify dominance hierarchies among males[18,19,92], the males in our lower density populations

consistently formed and defended territories (**Fig. 2**). While our experiment only examined one set of ecological and demographic conditions, it demonstrates a tractable field approach in which variables including food resources, defensibility of spaces, and demographic compositions are all easily tunable. There has been considerable interest in recent years in high-throughput measures of mouse social behavior in lab settings[17,18,93–95] and our study provides a blueprint for similar studies to be conducted under natural outdoor conditions.

What drives the difference that we saw in female space use across our trials (**Fig. 3**)? Space use in female mammals is often predicted by intra-sexual competition for food resources and nest sites[96], but resource availability and population density were identical across trials in our study. This suggests two non-mutually exclusive possibilities. First, there may be an innate difference in space-use behavior between C57 females and wild-derived outbred females. For example, this could be manifest as an increased tendency to explore irrespective of their social environment. Alternatively, female behavior may respond to the social conditions present in our trials, which differ in some key respects between the two genotypes. C57 females experience a society where everyone has very high genetic similarity, while outbred females experience a world with variation in relatedness. It is likely that mouse behavior in general might be sensitive to these parameters, though studies of social interactions in the lab frequently utilize single inbred strains of mice[97–99]. Female mice respond to variation in perceived relatedness between themselves and males[33,100,101] and could potentially attend to how they perceive males to be related to each other. In other words, it may be the case that females explore more in conditions when all social partners are genetically homogeneous but show more restricted space use when social partners are genetically heterogenous. In feral house mice, infanticide risk from both male and female conspecifics is thought to be a major driver of social behavior in females[102–104]. As a result, wild female house mice will aggressively defend space from other females[65,66,71,105,106] . In contrast, C57 mice have been bred to live in cages at high densities, especially among females, and this is associated with lower female aggression compared to wild mouse genotypes[40]. Differences in relative tolerance of other females may be a key driver of the observed differences in social organization between C57 and outbred females in this study. Understanding how innate behavioral differences among genotypes and emergent properties generated by social interactions shape mammalian societies is an exciting future direction that can be addressed using a similar rewilded mouse paradigm.

Male space use in rodents and other mammals is frequently linked to patterns of female space use[96,104]. Yet, despite differing patterns of female space use between genotypes, the male spatial and

social structures across genotypes were quite similar, highlighting that some aspects of social organization are relatively less sensitive to other features of a population's socioecology. Perhaps one of the most striking features of our study is the speed with which male-male social interactions decrease in frequency, especially among outbred males (**Fig. 2C**). Previous studies of wild mouse behavior have reported males will defend territories and attempt to monopolize spaces and exclude other males[25,29] and our data show this behavior is retained in male lab mice. The formation and consequences of dominance hierarchies among male mice have been the subject of recent study in the lab[19,92,107], but our results suggest that when given ample and defensible spaces male mice will tend to avoid interacting with others and form individual territories rather than a single integrated dominance hierarchy. Differences between territoriality and dominance behaviors remain poorly understood at the mechanistic level. Social dominance hierarchies and the establishment of territorial boundaries could be mediated by the same physiological and neurological mechanisms, or they might be mediated by distinct mechanisms. The process by which dominant males recognize known, tolerated subordinates may be fundamentally distinct from the process by which a territorial male characterizes intruders as well as neighbors versus strangers[108].

Across repeated trials, we identified differences in the higher-level social organizations of C57 lab mice and their wild-derived outbred counterparts (**Fig. 4**). Studies of social structures tend to come from idiosyncratic populations living in the wild, meaning that studies of social behavior in natural conditions are rarely replicated[53,109,110]. Studies of free-living populations are critically important, but this non-replicability makes understanding the specific genetic, neurobiological, ecological, and demographic factors influencing complex behavior challenging. The repeatability of social organization demonstrated here suggests that future work manipulating aspects of physiology or neural function in rewilded mice will offer a unique opportunity to study not just differences in individual behavior, but also how and whether specific behaviors reliably influence society.

Materials and methods

Animals

We examined two genotypes of *M. m. domesticus*, C57BL/6J (C57) and wild-derived outbred mice. We obtained C57 mice from The Jackson Laboratory (Bar Harbor, Maine, USA). We derived outbred mice from strains generated through distinct initial pairings of wild mice from Saratoga Springs, NY, USA, trapped by MJS in 2013[111]. These mice are descended from the same initial collection in Saratoga

Springs that gave rise to the wild-derived mouse strains SarA/NachJ (#035346), SarB/NachJ (#035347), and SarC/NachJ (#035348) available from the Jackson Laboratory. All experimental procedures conformed to guidelines established by the U.S. National Institutes of Health and have been approved by the Cornell University Institutional Animal Care and Use committee (IACUC: Protocol #2015-0060).

Study design and field site description

We conducted field work at Cornell University's Liddell Laboratory Field Station in Dryden, New York, USA from May 2020 to August 2020. Male (n = 10 per trial) and female (n = 10 per trial) mice were released into 0.056 hectare (38.1 m x 15.24 m) enclosures for 10-day observation periods before they were recovered using Sherman live traps (C57 trials: n = 4; outbred trials: n = 3). The walls of the enclosures were made from sheet metal and stood approximately 1.2 m tall and extended 1.2 m into the ground to prevent the mice from tunneling and moving between the enclosures. Each enclosure was covered with netting to prevent aerial predation, and loose gravel was spread along the interior perimeter of each enclosure to discourage digging near the walls. Three days prior to releasing mice into the enclosures, we trapped in and around the enclosures to capture and remove any small mammals or snakes from the enclosure. The enclosures contained a mixture of local perennial grasses and plant communities which were mowed to a height of ~5 cm prior at the start of each trial to maintain similar ecological starting conditions across trials.

Each enclosure contained eight identical resource zones constructed of PVC and 32-gallon storage totes (Rubbermaid, USA) arranged in a two by four grid pattern. Resource zones were covered with waterproof corrugated roofing material attached to a polyvinyl chloride (PVC) frame to shade the zones during the day and provide protection from rain. Resource zones had a single PVC entrance tube (50 mm diameter) through which the mice could freely enter or exit the tub. Each resource zone contained feeder towers containing food and water in excess (~50 grams of mixed bird seed and 2 liters of water). Several pieces of plastic lumber were added to provide environmental complexity within the resource zones. Each resource zone was equipped with a 15 cm RFID antenna connected to a centralized data acquisition unit (Biomark, Small Scale System, Boise, ID, USA). Antennas were placed directly beneath the floor adjacent to the PVC zone entrance tubes to increase the likelihood of capturing mouse entrances and exits from the resource zone. Scanning for RFID tags within the antenna range occurred at approximately 2-3 Hz continuously for 10 days. At least 24 hours prior to release in the enclosures, all subjects were placed into a stereotaxic frame (Kopf Instruments, Tuhunga, CA, USA) and briefly

anesthetized with isoflurane (3-5%). Mice were subcutaneously implanted with dual passive integrated transponder (PIT) tags (BioMark, Boise, ID, USA) in the dorsal flank and periscapular region.

After the 10-day observation period, >50 live-catch traps (H.B. Sherman, Tallahassee, FL, USA) were baited with sunflower seeds and a moistened cotton ball and placed in a grid pattern in the enclosures in the evening (20:00-22:00 hours) and were checked for occupancy the following morning (07:00-09:00 hours). Trapping continued until all the mice were recovered or identified as deceased or missing (a conclusion reached if there were no RFID reads in the enclosure for a 24-hour period after 3 days of trapping). The trap locations were recorded, and the individual identities of the mice were confirmed using a handheld RFID reader (BioMark, HPR Lite).

RFID data analyses

We conducted two types of RFID analyses focusing on (1) when and at what zone each mouse was detected and (2) estimating how long they spent at each zone. In the first set of analyses, we examined whether or not mice visited a given zone during each day of the trial (**Fig. 2A; Fig. 3A-C; Fig. S1G; Fig. S2A&F; Fig. S3A**). We estimated the minimum distance travelled using consecutive transitions in the RFID reads between antennas in different resource zones and the known spatial layout of the enclosure (**Fig. S3D**). To assess the degree to which resource zones were exclusively accessed by individual mice, we examined the percent of same-sex sourced RFID reads in zones for each mouse (**Fig. 2B**).

In our second set of analyses estimating the durations of time mice spent in zones, we first examined the time elapsed between consecutive RFID read events for each mouse within each resource zone (the RFID inter-read interval). We found that the distribution of all RFID inter-read intervals was heavily skewed (min = 1s, median = 1s, mean = 16.4s, max = 32,683s; **Fig. SE**). We grouped RFID reads for each mouse for each zone into visitation bouts using a 139 second (the cut-off for capturing 99% of all the within-mouse inter-read interval values within a single zone) sliding window method such that an RFID read which occurred within 139 seconds of the previous read extended the visit bout duration up to that read. Transitions between zones automatically ended and started bouts in the first and second zones, respectively. We assigned isolated single RFID reads (with no other reads within 139 seconds before or after the focal read) a visit bout duration of 1 second. Using this dataset, we estimated the time mice spent in resource zones (**Fig. S2B**). Mice were defined as participating in a spatiotemporal association bout when two or more mice had overlapping resource zone visit bouts within the same zone

(see **Fig. S1F** for a graphical schematic of the approach). We used the spatiotemporal association bout dataset to estimate the duration and frequency of male-male (**Fig. 2C**), female-male (**Fig. S2E**), and female-female (**Fig. S3F**) dyadic association bouts. We omitted a subset of animals from a subset of days for all spatial and social analyses when they received no reads over multiple days and were presumed dead (see **Table S1** for details).

Priority Access Score calculation

Priority access scores were calculated separately for male and female mice within a trial. First, we calculated the time a given mouse (*M*) occupied a resource zone (*Z*) as a percentage of the total time that zone was occupied by same-sex conspecifics on a given day (*D*).

$$Occupancy_{M,D,Z} = \frac{time_{M,D,Z}}{\sum_{m=1}^{10} time_{m,D,Z}}$$

Next, we calculated a daily Capture Score by summing the Occupancy values for all available zones. Mice that did not have an occupancy value of greater than 0.5 (in other words, a majority share of the time spent in any particular zone), were penalized by subtracting 1 from the final Capture Score. The penalty indicates that on a given day, a mouse failed to capture any of the zones that mouse visited.

$$Capture\ Score_{\ M,D} = \begin{cases} \sum_{z=1}^{8} Occupancy_{M,D,z} & \text{if } \exists\ Occupancy_{M,D,z} > 0.5\ \forall\ z = 1, \dots, 8 \\ \sum_{z=1}^{8} Occupancy_{M,D,z} - 1 & otherwise \end{cases}$$

To see how access to zones changed over time, we took the cumulative sum of an individual's Capture Score ordinally across each day of the trial to derive an evolving Priority Access Score on each day of the trial.

$$Priority\ Access\ Score_{M,D} = \sum_{d=1}^{D} Capture\ Score_{M,d}$$

As an example, if one male (Male A) occupied a single resource zone every day of the trial for 4 hours a day, while another male (Male B) accessed only that same zone for 1 hour per day, and the zone was visited by no other mice, each mouse would yield the following values. Male A's daily Capture Score would equal 0.8, (because he controlled 4 out of 5 hours), while Male B's daily Capture score would equal -0.8 (because he controlled 1 out of 5 hours and received a one-point penalty for not controlling any zones). If this pattern of visitation remained unchanged for all 10 days, then Male A's final Priority Access Score would equal 8, while Male B's Priority Access Score would equal -8. The Priority Access Score value thus provides a temporally evolving measure that captures the dynamics of territory formation, maintenance, and collapse (**Fig. S2D; Fig. S3B**).

Male spatiotemporal overlap and win/loss at home and away

We used the zone visit bout and spatiotemporal association bout dataset to investigate the degree to which territorial and intruder males avoided spatiotemporal overlap. Across all zones for the last 6 days of the trial, we independently calculated the total time territorial and intruder males spent in zones and the amount of that time that was spent in spatiotemporal overlap. We derived null expected values of territorial and intruder male spatiotemporal overlap ((total territorial male hours in zones * total intruder male hours in zones) / (24 hours * 6 days * 8 zones)) and examined the percent difference between the null expectation and the observed time territorial and intruder males overlapped. We chose the last 6 days of the trials for this analysis as territorial relationships were clearly established by this point in the trial.

Next, we used the RFID and spatiotemporal association bout data set to estimate the dynamics of male spatiotemporal overlap and territorial intrusion by identifying clusters of male overlap events when the number of males within a zone went from 1 to 2 to 1 consecutively in time across events. In other words, we identified instances in the dataset where a single male within a zone was joined by an additional male, followed by one of the males leaving. We identified the male participants as either the territory holder of that zone or as an intruder based on whether the focal male had captured >50% of all of the male-derived RFID reads within the zone in which the male spatiotemporal overlap event occurred. We derived 'win' and 'loss' rates both within and away from a home territory for each male participant on each day of the trial by assigning the remaining male and the male that left as the 'winner' and 'loser' of the event cluster, respectively (**Fig. 2**).

Female spatiotemporal overlap and cagemate associations

Using the known cage mate relationships between individual females, we examined the total time female mice spent in spatiotemporal association with cage mate (sibling) versus non-cage mate females (**Fig. S3G**). Based on the number of cage mates and non-cage mate female social partners available on each day of the trial (which fluctuated for some trials based on mice disappearing from the trials; see **Table S1**), we calculated a daily null value of the expected time spent with cage mates and the percent observed value deviation from the expected separately for each female (**Fig. 3D**).

Social network analyses

We used the spatiotemporal association bout data set arranged in a group by individual matrix to construct social networks. Daily weighted adjacency matrices were derived from a Simple Ratio Index calculation based on counts of binary participation in spatiotemporally overlapping mouse zone visit bout events (where 0 indicates that individuals were never in the same spatiotemporal association bout and 1 indicates that individuals shared all spatiotemporal association bouts**; Fig. S1F**) using the *asnipe*[112] package in R 4.1.2 (R Development Core Team). All networks were constructed using the weighted matrices and the *igraph*[113] package in R. Vertex sizes in our network reflect the node edge strength, or the sum of all connected edges to the node, while edge widths reflect the edge weight. If an animal died or went missing, we no longer included it in a given trial's daily network[114,115] (see **Table S1**).

Statistical analyses

We built mixed effects models using R 4.1.2 (R Development Core Team) and the R packages *lme4*[116], *lmerTest*[117], and *emmeans*[118] to examine relationships between predictor and response variables. We include the full statistical test and model outputs in the Supplementary Material for all analyses. Most analyses are conducted with a repeated measures design where data is examined for each day per mouse or other relevant unit as appropriate using mixed effects models. Across models we considered random effects of trial, mouse, and/or time in the trial. We included relevant random intercepts and random slopes in our models as appropriate – random slopes of time were generally included, but we occasionally needed to simplify random effects structures when necessary to avoid singular fits. All model formulae, including random effects structures, are explicitly reported in the Supplemental Material. For testing for unimodality, we used the *multimode*[119] package with "ACR" method[120].

Graphing was done in R using the package *ggplot2*[121] and in GraphPad Prism 9.3 (www.graphpad.com). We report all means ± standard error (SE), unless otherwise stated, and consider all values statistically significant when $P < 0.05$.

Declarations

Consent for publication

All authors have read and approved this manuscript for publication.

Acknowledegments

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Author contributions

1) Conceptualization, 2) Study Design, 3) Methodology Design, 4) Field Work, 5) Data Curation & Code, 6) Data Analysis, 7) Figure Creation, 8) Writing – Original Draft, 9) Writing – Review & Editing, 10) Supervision, 11) Funding Acquisition. CCV: 1,2,3,4,5,6,7,8,9,10,11. MNZ: 6,9. DDS: 4,9. CHM: 4,9. SXH: 6. MKA: 6. AMG: 6. MSC: 4. AJM: 4,9,10,11. MJS: 1,2,4,6,7,8,9,10,11.

Code and data availability

All statistical output tables are included as supplementary files. Code and scripts for reproducing figures and analyses are available on GitHub (https://github.com/calebvogt/2020_CV_LID). All data files are available on Zenodo (https://doi.org/10.5281/zenodo.6425497).

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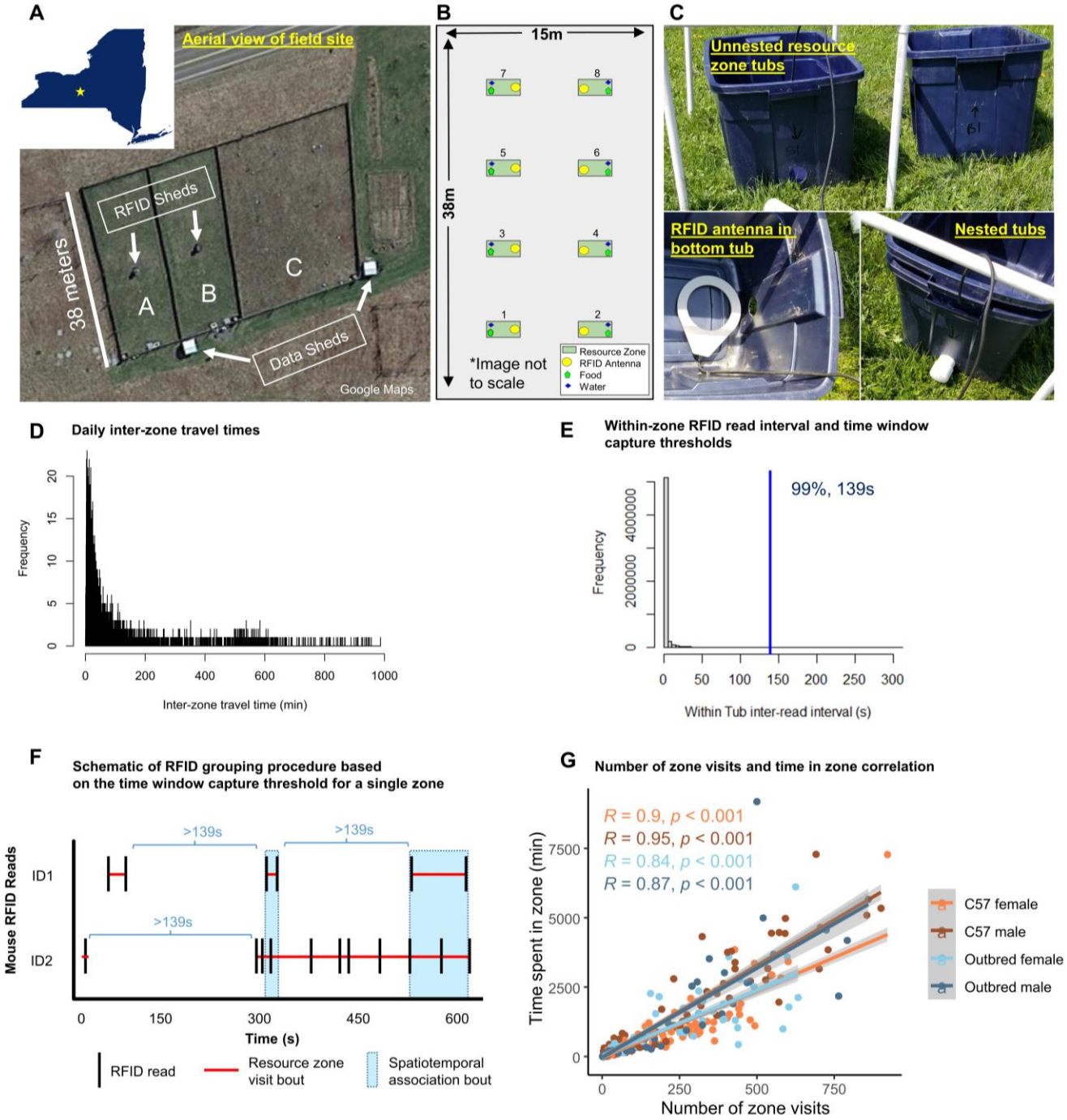

Figure S1: Field site setup and RFID duration bout window selection

(A) Satellite image of the field enclosures showing the position of the data sheds housing the computer for downloading RFID data from the central RFID sheds. **(B)** Graphical schematic of the Alpha and Bravo enclosures indicating the resource zone layouts. **(C)** RFID monitoring of the resource zones. Two storage totes were nested with a RFID antenna placed between them, beneath the entrance tunnel to prevent mice from directly contacting the antenna and wire. **(D)** Histogram of the daily inter-zone travel times for all mice for all days. **(E)** Histogram of the within zone inter- RFID read intervals and the 139 second threshold capturing 99%

of all inter-RFID read intervals within the same zone which was used to group RFID reads into resource zone visitation bouts (see Methods). **(F)** Schematic of the time window capture threshold grouping procedure to determine resource zone visit bouts and spatiotemporal association bouts using mock RFID data and the time window capture threshold (139 s). **(G)** Correlation of estimated duration spent in each zone and the number of visits to that zone for all sex and strain categories.

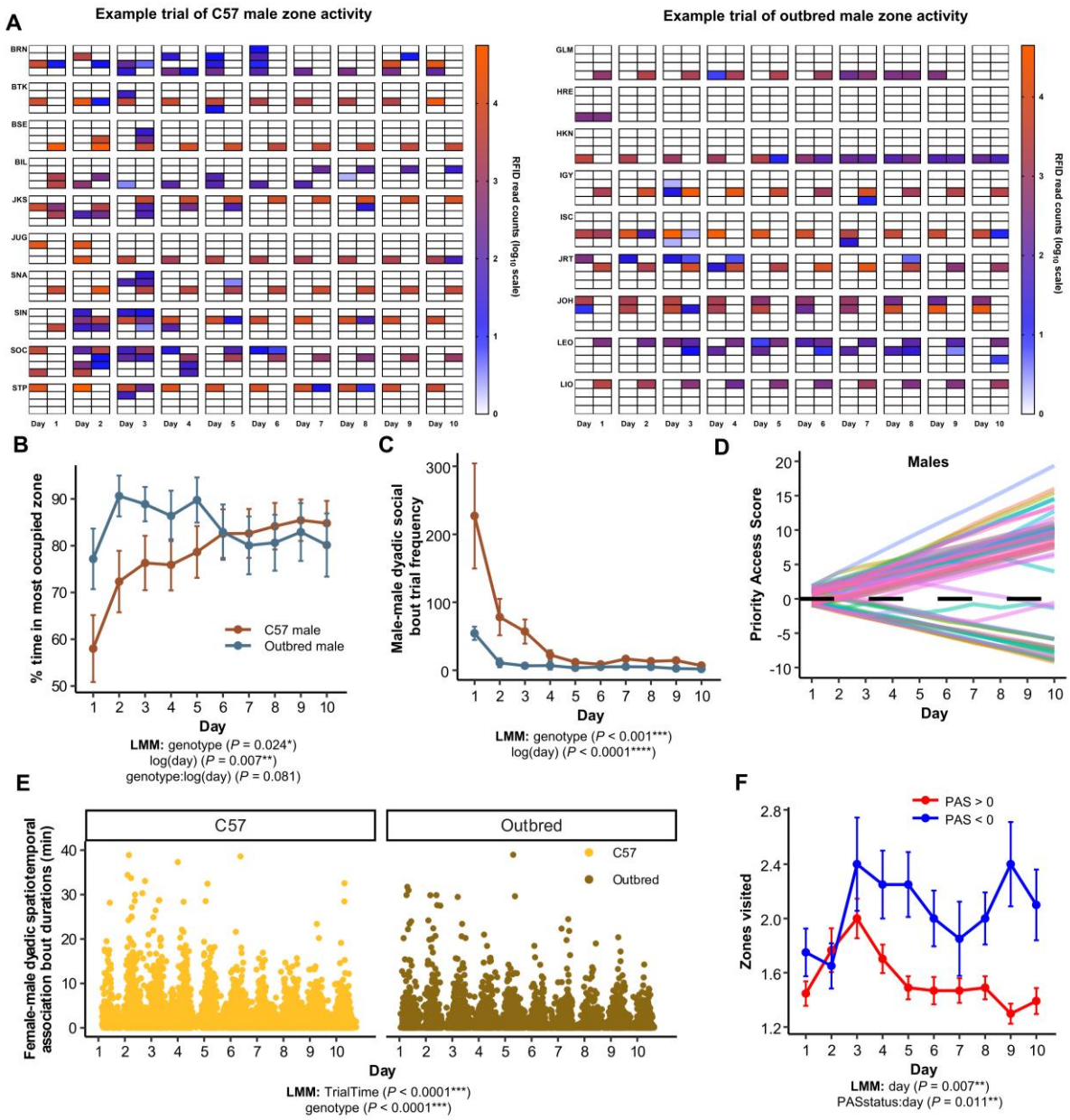

Figure S2: Territory establishment in C57 and outbred male mice

(A) Schematic of the resource zone locations (colored boxes) within the field enclosures (2x4 grids) showing patterns of male resource zone usage (rows) for an example C57 (left) and outbred (right) trial across 10 days of activity (columns). White boxes indicate resource zones that were not visited by the focal individual. **(B)** Percentage of the total time a mouse was observed across all zones spent in a mouse's top occupied zone (resource zones rank ordered by mouse occupancy time). **(C)** Male-male social grouping events fell rapidly over time and were less frequent in outbred trials compared to C57 trials. **(D)** Over time males tend to either gain priority access to resource zones (i.e., are territorial) or do not (floater males). Y-axis shows the evolving daily Priority Access Scores over 10 days of observation for males of both genotypes (See Methods for additional details on calculation of the daily PAS value). **(E)** C57 mice had longer male-female social grouping bout durations overall. For visualization purposes, the y-axis is cut off at 40 (n = 15,891 events shown out of

15,898 total events). **(F)** Daily zones visited were lower for high status males (Day 10 PAS > 0) than for low status males (Day 10 PAS < 0).

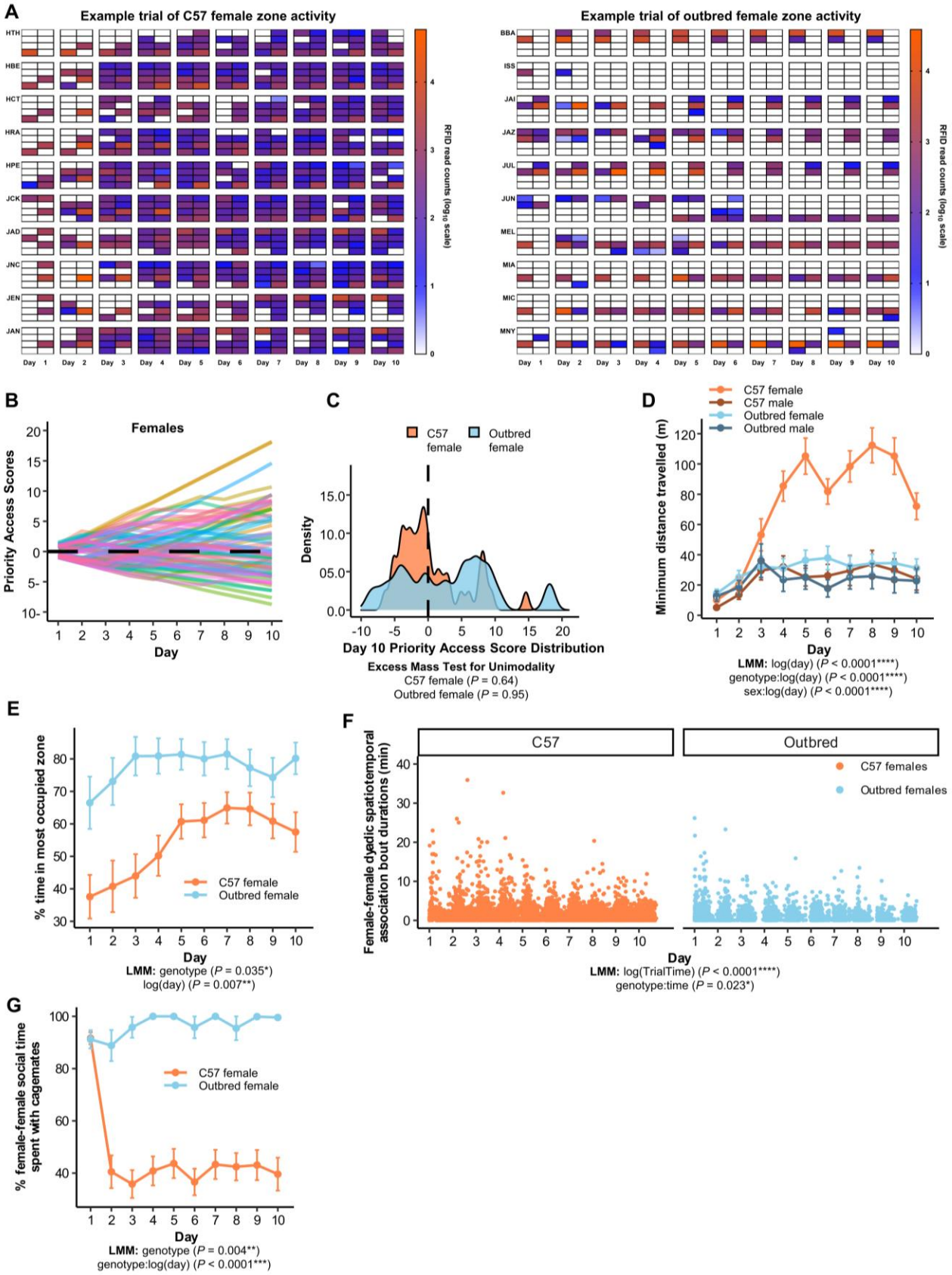
A
Example trial of C57 female zone activity
Example trial of outbred female zone activity
RFID read counts (log10 scale)
B
Females
Priority Access Scores
Day
C
C57 female
Outbred female
Density
Day 10 Priority Access Score Distribution
Excess Mass Test for Unimodality
C57 female (P = 0.64)
Outbred female (P = 0.95)
D
C57 female
C57 male
Outbred female
Outbred male
Minimum distance travelled (m)
Day
LMM: log(day) (P < 0.0001****)
genotype:log(day) (P < 0.0001****)
sex:log(day) (P < 0.0001****)
E
% time in most occupied zone
C57 female
Outbred female
Day
LMM: genotype (P = 0.035*)
log(day) (P = 0.007**)
F
C57
Outbred
C57 females
Outbred females
Female-female dyadic spatiotemporal association bout durations (min)
Day
LMM: log(TrialTime) (P < 0.0001****)
genotype:time (P = 0.023*)
G
% female-female social time spent with cagemates
C57 female
Outbred female
Day
LMM: genotype (P = 0.004**)
genotype:log(day) (P < 0.0001***)

Figure S3: Distinct patterns of space utilization in C57 females

(A) C57 females extensively explore the available resource zones over the course of the trial compared to outbred females. Example full trial data of female zone use in C57 (left) and outbred (right) mice. Schematic of the resource zone locations (colored boxes) within the field enclosures (2x4 grids) showing patterns of zone usage for animals (rows) across 10 days of activity (columns). White boxes indicate resource zones that were not visited by the focal individual. **(B)** Daily Priority Access Scores over 10 days of observation for female mice. **(C)** Distributions of Day 10 Priority Access Scores for female mice are not multi-modal (excess mass test for unimodality from the multimode package), indicating decreased or inconsistent monopolization of resource zones amongst females. Higher scores indicate the extent to which a mouse maintained majority access over one or more resource zones relative to same-sex conspecific competitors (see Methods for details). **(D)** C57 female mice (n=40) differed from C57 male (n=40) and outbred male (n=29) and female (n=30) mice in their estimated minimum distance travelled over the course of 10 days. **(E)** Outbred females spent more time in their most occupied zone than C57 females. **(F)** Female-female social grouping bout durations over time. For visualization purposes, the y-axis is cut off at 40 (n = 11,928 events shown out of 11,933 total events). **(G)** Outbred females spent nearly all of their female-female social time with cage mates after day 2, in contrast with C57 females who generally spent less than half of their female-female social time with cage mates.

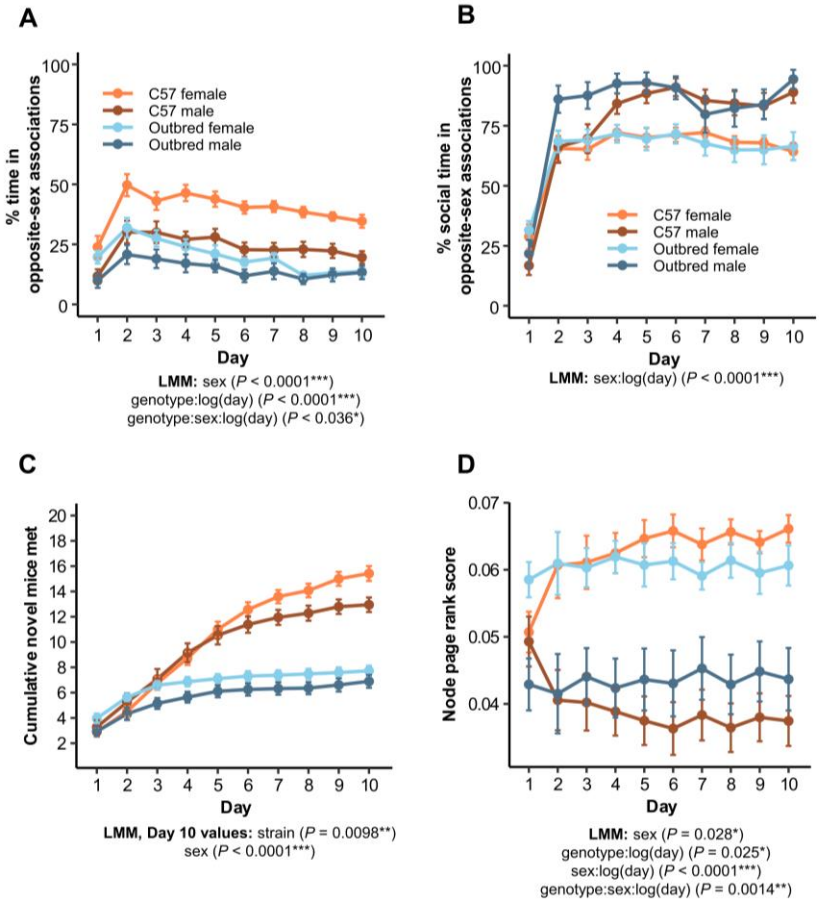

Figure S4: Repeatable differences in C57 social structure and network level properties over time
(A) Percentage of total recorded observation time spent in opposite-sex associations is higher in C57 mice over the course of the trial. **(B)** Percentage association time spent in opposite sex associations is higher in males than females for both genotypes. **(C)** C57 mice met a majority of the available novel social partners by the final day of the trial, while outbred mice did not. **(D)** Both C57 and outbred females exhibited high page rank scores relative to males of either genotype, indicating that females serve as major social connections through the network in both genotypes.