Temporal dynamics of social hierarchy formation and maintenance in male mice

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Dominance hierarchies emerge when individuals must compete for access to resources such as food, territory or mates. Here, using traditional and network social hierarchy analysis, we show that 10 groups of 12 male laboratory CD1 mice living in large vivaria consistently form extremely linear dominance hierarchies. Within each hierarchy we determine that every individual mouse has a unique social rank and behaves with a high degree of consistency in their agonistic behaviour towards other individuals. Using temporal pairwise comparison Glicko ratings and social network triangle transitivity measures, we demonstrate that these hierarchies emerge rapidly, and that initial aggression is not predictive of later dominance. We also show that groups vary in how unequally power is distributed over time as social networks stabilize. Our results demonstrate that an ethologically relevant housing paradigm coupled with extensive behavioural observations provides a strong framework for investigating the temporal patterning of mouse dominance hierarchies and complex social dynamics. Furthermore, the statistical methods described establish a strong basis for the study of temporal dynamics of social hierarchies across species.
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Social dominance occurs when one individual repeatedly and consistently yields towards another individual's agonistic behaviour, leading to a de-escalation rather than escalation of future aggression within that relationship (Drews, 1993). A dominance hierarchy emerges when most relationships within a social group are organized such that more dominant individuals consistently induce yielding responses in more subordinate individuals (Chase, 1982b). Hierarchies form when there is competition for resources such as access to mates, food or territory. Recognizing and adhering to a social rank may be beneficial by preventing the need for constant conflict and risk of injury (Chase & Seitz, 2011). First described by Schjelderup-Ebbe (1922) based upon his observations of domestic fowl forming a 'pecking order', dominance hierarchies are now one of the most well-studied forms of social organization, occurring naturally in diverse species, including fish, reptiles, birds, mammals, primates and humans (Chase & Seitz, 2011). Dominance hierarchies also emerge readily in species studied in the laboratory such as cichlids (Fernald & Maruska, 2012; Oliveira & Almada, 1996), crayfish (Issa, Adamson, & Edwards, 1999) and chickens (Chase, 1982a).

Traditionally, the study of social behaviour in laboratory mice has been limited to brief dyadic interactions occurring in a context separate from the home-cage environment (Brodkin, 2007; Crawley, 2007; Kas et al., 2014). Although these tests reveal behaviour characteristics of individual mice and the relationship between two individuals at a given point in time, they do not provide information about how relationships develop over time or how relationships are adjusted within a large social network. Dominance in pairs of mice is usually assessed with dyadic tube-tests (van den Berg, Lamballais, & Kushner, 2015; Curley, 2011; Wang et al., 2011), food, sex or other reward competition tests (Benner, Endo, Endo, Kakeyama, & Tohyama, 2014; Jupp et al., 2015; Nelson, Cunningham, Ruff, & Potts, 2015) and aggression tests (Branchi et al., 2013; Ginsburg & Allee, 1942). Problematically, results in these social contexts do not necessarily relate to overall social dominance within a larger group context where relationships are embedded (Chase, 1982b). Studies that have examined social dominance in groups of male laboratory mice have limited their scope to the emergence of an alpha male rather than determining finer details regarding the rank order of all individuals (Ely & Henry, 1978; Lewejohann et al., 2009). Moreover, previous studies of social

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dominance in the laboratory have limitations such as small group sizes, short duration of observations and few replicated groups (Arakawa, Blanchard, & Blanchard, 2007; Ely & Henry, 1978; Lewejohann et al., 2009; So, Franks, Lim, & Curley, 2015).

Our laboratory has developed a novel paradigm for the study of the social behaviour of group-living laboratory mice that addresses these shortcomings. We house groups of laboratory mice, *Mus musculus,* for several weeks in a large vivarium that mimics the natural burrow system of the ancestral species (Berry, 1970). The environment comprises a below-ground level of interconnected nestboxes and above-ground levels that contain food, water and environmental enrichment (So et al., 2015; Supplementary Fig. S1). Since *Mus musculus* are characterized by high male reproductive skew with high intermale competition (Crowcroft, 1973), we used all-male groups in the current study. We collected live observational data from 10 separate social groups and used advanced statistical techniques to investigate whether male outbred laboratory mice consistently form linear dominance hierarchies. We then examined the temporal dynamics of mouse social hierarchies, determining how hierarchies are established, how inequitable the distribution of power within the dominance network is, and how stable hierarchies are over time. We believe that this work provides a strong conceptual framework for the study of complex social dynamics within the laboratory that has implications for our understanding of behavioural parameters relevant to social relationships in natural contexts.

METHODS

Animals and Housing

A total of 120 male outbred CD1 mice aged 7 weeks were obtained from Charles River Laboratories (Wilmington, MA, U.S.A.) and housed in groups of three in standard sized cages ($27 \times 17 \times 12$ cm) with pine shaving bedding. All mice were assigned individual IDs and marked accordingly by uniquely dying their fur with a blue, nontoxic, nonhazardous marker (Stoelting Co., Wood Dale, IL, U.S.A.). These marks last for up to 12 weeks, so one application enabled unique individual identification throughout the 23-day study. At the age of 9 weeks, mice were randomly assigned to social groups (cohorts) consisting of 12 males. In each cohort, six males had no previous experience of any other male in the cohort and six males had previously been housed with only one other male that was in that cohort. Each individual was weighed and placed into a large custom-built mouse vivarium (150×80 cm and 80 cm high; Mid-Atlantic, Hagerstown, MD, U.S.A.; see Supplementary Fig. S1). Vivaria were constructed as described in So et al. (2015), consisting of multiple shelves, nestboxes and a metal backboard containing multiple holes for air circulation. Mice could explore and access each shelf and cage via ramps and tunnels. Standard chow and water were provided ad libitum at the top of the vivarium. Multiple enrichment objects such as plastic igloos and round tubes were also provided. Pine shaving bedding was used to cover the shelves and nestboxes in each vivarium. Animals were put into the vivarium just prior to the onset of the dark:light cycle on day 1 of the study and were not disturbed for the duration of their housing in the vivarium (21—23 days). All subjects were housed in the Department of Psychology at Columbia University, with constant temperature (21—24 °C) and humidity (30—50%), and a 12:12 h light:dark cycle with white light (light cycle) on at 2400 hours and red lights (dark cycle) on at 1200 hours. All procedures were conducted with approval from the Columbia University Institutional Animal Care and Use Committee (IACUC Protocol No. AC-AAAG0054) and are in concordance with ASAB/ABS guidelines. At the end of the experiment all animals were euthanized via decapitation, with each individual's brain and blood being stored for future analyses.

Behavioural Observations

Live behavioural observations commenced on the first day of group housing in the vivarium and continued for 21—23 days per vivarium (see Table 1). Observations were conducted for 1—3 h per day during the dark cycle, with the majority of observations occurring in the first 4 h after the onset of the dark cycle. The mean ± SD total observations conducted per vivarium was 43.05 ± 6.29 h (range 34—52 h). Using all-occurrence sampling, trained observers recorded all occurrences of the behaviours listed in the ethogram in Supplementary Table S1 that occurred between two animals within each 1 h observation period. Often several behaviours co-occur within each aggressive contest. Each contest lasted 1—20 s (typically 5—10 s). Behaviours were recorded with the following priority: fighting, chasing, mounting, subordinate posture, induced-flee. For instance, if one animal fought another animal that responded by fleeing, this would be recorded as a fighting event only, as fighting takes priority over the co-occurring induced-flee. If an animal fled when approached but was not attacked by another animal, then this would be recorded as induced-flee. For each behavioural event, the subject directing the behaviour, the recipient of the behaviour, and the time and location within the vivarium was recorded. Individuals that directed fighting, chasing or mounting were considered winners of each interaction. Individuals that exhibited subordinate posture or induced-flee towards another subject were considered losers of each interaction. If there was no clear winner, then the event was recorded as a tie. Each subject would only receive one win (or one loss) per aggressive interaction even if several behaviours (e.g. chase, fight, subordinate posture) co-occurred during that interaction. This was done so as not to inflate the total number of wins and losses per individual. Aggressive interactions were considered to have ended when each individual separated and engaged in different behaviours such as self-grooming, social investigation of other animals, nest building, feeding, etc. All data were documented live via Google survey on Android devices. All observers were trained to >90% reliability.

Statistical Analysis

All statistical analyses were undertaken in R v.3.2.2 (R Core Team, 2015). The total frequency of wins and losses accrued by each individual was aggregated into separate frequency win/loss sociomatrices for each cohort, with winners in rows and losers in columns. A binarized 1/0 win/loss sociomatrix was derived from each frequency win/loss matrix. Following the methods of Appleby (1983), for each cell of the frequency win/loss matrix we assigned a 1 to individuals in rows that won absolutely more often against individuals in columns and a 0 to individuals in rows that lost absolutely more often to individuals in columns. If individuals were tied, both individuals received a 0.

Using the frequency win/loss sociomatrices, we calculated the following measures of the strength of the social hierarchy: (1) Landau's modified h' evaluates the extent to which individuals in a hierarchy can be linearly ordered (de Vries, 1995). It ranges from 0 (no linearity) to 1 (completely linear), with the significance of h' determined by performing 10 000 two-step randomizations of the win/loss frequency sociomatrix and comparing the observed h' against a simulated distribution of h'. (2) Directional consistency (DC) assesses the degree to which all agonistic interactions in a group occur in the direction from the more dominant individual to the more subordinate individual within each relationship. It is