Friend virus severity is associated with male mouse social status and environmental temperature

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Pathogen virulence is highly variable within populations, and although many factors contributing to virulence differences are known, there is still much variation left unexplained. Identifying and characterizing environmental conditions associated with different virulence levels is therefore an important undertaking in infectious disease research. One factor considered to be a major determinant of overall health and susceptibility to disease in social animals is social status. Health differences associated with social status are thought to be caused by different levels of chronic stress in higher- versus lower-status individuals. There is considerable evidence that these effects extend to the standing immune profile and that social status directly influences susceptibility to pathogens. Here we examined the association between dominance status in male wild-derived house mice, *Mus musculus*, and susceptibility to Friend virus complex in the context of seminatural populations with intense male–male competition and no predation. Due to an interruption in our facility’s heating system, we were unexpectedly presented with the opportunity to assess how reduced ambient temperature influences the association of host social status and pathogen virulence. Environmental temperature has been implicated as a contributor to pathogen virulence, giving us a unique chance to examine its role in a previously unexamined pathogen system, while the added context of social status can expand our understanding of how the interaction of different environmental conditions affects virulence. We found that pathogen virulence and replication were lower in socially dominant hosts compared to nondominant hosts. When temperature was reduced, cool enclosure-housed dominant males were more susceptible to infection than their warm enclosure-housed counterparts. The mechanistic underpinnings that link infectious disease and social status remain difficult to disentangle from their associated factors, but this study opens the door for future experiments using a novel approach in the most well-studied mammalian model available.

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temperature in response to rhinovirus infection (Foxman et al., 2015). Alternatively, environmental temperature may also have indirect effects on pathogenic infection through changes in host sociality and behaviour, as seen in a fish (Bartolini et al., 2015).

In social animal models that allow for manipulation of social status, relative social position itself has been shown to cause changes in immunological traits. In female rhesus macaques, Macaca mulatta, social status is associated with differing immune profiles, with high-status individuals interpreting as having a stronger antiviral immune profile and low-status individuals displaying a more antibacterial profile. Importantly, these differences can be reversed by altering individual social status, suggesting a causal relationship (Snyder-Mackler et al., 2016). The rhesus macaque system has also revealed that changes in social status cause regulatory changes in many genes in peripheral mononuclear blood cells, particularly those involved in interleukin signalling, T-cell activation and inflammation (Tung et al., 2012). It is worth noting that these studies do not involve experimental infections, instead examining standing immunity and ex vivo assays of the response to LPS. In mice, monitoring and manipulating male social status has been recently explored and found to have profound direct regulatory effects (Nelson, Cauceglia et al., 2013; Nelson, Colson et al., 2013; Nelson et al., 2015) and is reversibly associated with changes in oxidative stress rate and metabolic gene expression (Cauceglia et al., 2020). Despite the extensive literature establishing the effects of low social status leading to poorer health and higher infectious disease susceptibility in social animals (Cohen et al., 2007, 1997; Hawley et al., 2007; Schmidt et al., 2008), experimental systems capable of directly examining the mechanistic links are few. Utilizing seminatural enclosures of house mice, Mus musculus, allows for both the careful monitoring and manipulation of the social environment along with the use of live, well-characterized pathogens. Such a system may provide the needed capabilities for experimenters to rigorously conduct such investigations.

Here, we tested the hypothesis that social status is associated with infectious disease outcome. We expected that dominant males would better withstand infection, either reflecting greater vigour required to maintain dominance, or a causal influence of social status on immune function. We establish the association between social status and infectious disease outcomes in a scientific work horse, the house mouse, recapitulating the pattern seen in other social species and confirming our prediction that dominant male mice have less virulent infections associated with reduced pathogen replication. This was achieved by using seminatural enclosures of mice infected with a mouse-specific retrovirus (Friend virus complex, FVC), which has previously been used to study antiviral immunity and pathogen evolution (Cornwall et al., 2018; Hasenkug & Chesebro, 1997; Kubinak & Potts, 2013; Kubinak et al., 2013). Seminatural mouse populations have been used to successfully answer diverse questions (e.g. Gaukler et al., 2016; Morris et al., 2017; Ruff et al., 2013; Schmid-Holmes et al., 2001; Schrading et al., 2009), with recent investigations exploring their broad utility in understanding environmental effects on immune function and behaviour (Cope et al., 2019; Leung et al., 2018; Lin et al., 2020; Lopes et al., 2016). The seminatural enclosures used here allow for the delineation of male mouse social status by measuring the ability of males to control a territory by excluding competitors. Males that control territories are thus considered to be socially ‘dominant’ (Meagher et al., 2000; Ruff et al., 2013). An unexpected disruption of enclosure temperature during these experiments allowed us to test the hypothesis that environmental temperature influences FVC virulence and replication and to uncover potential interactions with social status effects. Because of previously observed associations of low temperature with more virulent infections, a similar pattern in this study was predicted. Indeed, the association between social status and virulence was lost in cooled enclosures, while dominant males showed increased FVC replication in cooled enclosures. The association between social status and susceptibility to infection reported here, combined with the ability to manipulate social status in seminatural mouse enclosures recently reported (Cauceglia et al., 2020), will enable future experiments to elucidate the underlying mechanisms between social status and infectious disease. Furthermore, it may lead to novel interventions in infectious disease mitigation and treatment in diverse settings including captive animal populations, natural ecological systems and human societies.

METHODS

Animals

Animals were kept under a 12:12 h light:dark cycle and provided with ad libitum food (Teklad rodent chow, Envigo catalogue no. 2018) and water throughout. In order for dominance hierarchies to be established in seminatural enclosures, it was necessary to utilize wild-derived mice (M. musculus), as standard inbred mouse lines do not exhibit normal behaviours required to compete and defend territories (Nelson, Cauceglia, et al., 2013; Nelson, Colson et al., 2013). Animals in this study were from the 19th and 20th generations of the outbred colony previously described (Meagher et al., 2000). This colony was maintained and outbred in standard laboratory breeding cages, with pedigree data available for all litters. Standard cages were 19.56 × 30.91 × 13.34 cm and contained a maximum of five mice per cage. Each cage was furnished with paper chip bedding, with shredded paper provided as a nesting material for enrichment. Rooms that housed mice were kept within approved temperature and humidity ranges (68–79 °F or 20–26 °C and 30–70% humidity, respectively). Mice were chosen from 47 litters born from 45 breeding cages (60 males and 60 females total). Litters were chosen based on below criteria, with up to two brothers and two sisters randomly selected therein. Same-sex siblings were then separated to populate three pairs of concurrent replicate enclosures (6 enclosures, 10 males and 10 females each). Assignments maximized the number of unique grandparents (minimize cousin level relatedness) and balanced degree of relatedness overall. Litters were also chosen to minimize postpuberty age variation at their release into enclosures (mean ± SD = 14.6 ± 4.4 weeks). All of this was assisted by the spread of our replicates occurring in pairs over two generations.

Seminatural Enclosures

Seminatural enclosures were approximately 30 m², divided into six subsections by hardware cloth that mice could climb and patrol. Four of these subsections, deemed ‘optimal’, contained nestboxes enclosed in an opaque box containing an ad libitum food source. The two remaining 'suboptimal' subsections contain light-exposed nestboxes separated from the subsections' own ad libitum food sources. Optimal subsections are preferred nesting areas for females, and males will fight to acquire and maintain control of such territories (Fig. 1b, territories 1–4) or be relegated to the suboptimal territories (Fig. 1b, territories 5 and 6). Each of the six enclosures was founded by 10 male and 10 female mice, reflecting population densities found in natural human architecture-associated populations (Bronson, 1979). An unexpected disruption of the building's heating system resulted in temperatures falling below approved housing conditions ('warm enclosures' – 68–79 °F or 20–26 °C) in the facility while two of the six enclosures were being conducted. Unsuccessful attempts were made to correct this problem by University maintenance teams, and lower
temperatures (‘cool enclosures’ = −60–68 °F or −16.5–20 °C) persisted for the duration of two replicates. These temperature ranges were recorded from a single stationary thermometer and humidity meter.

Study Design

All mice were released into seminatural enclosures and allowed to acclimate to the more natural social competition conditions, and for males to establish dominance hierarchies (Fig. 1a, b; day 1). On the 13th day after initial release, all mice were captured and individually housed in standard cages for less than 1 h. Mice were then infected with Friend virus complex and re-released into their original enclosures and all previously established territoriality quickly returned (Fig. 1b). Mice were recaptured 12 full days postinfection and euthanized for sample collection and virulence measurement (Fig. 1a; day 25). In all cases, dual-method euthanasia was performed by CO2 asphyxiation followed by cervical dislocation. Seminatural enclosures were checked daily for animal health and food and water levels. Any mouse observed to be exhibiting distress (e.g. excessive wounding or bleeding, immobility or hunching) during the experiment was removed from the enclosure.

Figure 1. Graphical design summary and male dominance outcomes from one of six replicate enclosures. (a) On day 1, 10 males and 10 females were PIT-tagged and released into large enclosures divided into six territories of two types: ‘optimal’ (1–4), which included dark and easily defendable nestboxes; ‘suboptimal’ (5, 6), which were more exposed. Each food source (black cylinder) was continuously monitored by a PIT tag reader. On day 13, all mice were briefly captured, infected via IP injection with Friend virus complex (FVC) and returned to the same enclosure for another 12 days. On day 25 all mice were again captured and euthanized for sample collection. (b) Territoriality, as measured by the daily percentage of male PIT tag reads, was acquired for each male (M-01–M-10) in each of the six territories, arranged to reflect enclosure layout. A male was considered ‘dominant’ if he retained >80% of the PIT tag reads prior to infection (dotted red line). Male M-08 was dominant in both territories 1 and 2, male M-01 was dominant in territory 3 and male M-10 was dominant in territory 4. All other males co-inhabited suboptimal territories 5 and 6 but invaded and challenged dominant males frequently.
euthanized for humane considerations and assessed for spleen rupture. Mice that failed to survive the 12-day infection were censored from spleen mass and viral replication analysis. As a control, one enclosure was established as described above, except instead of being infected with FVC on day 13, mice were sham-infected with phosphate buffered saline.

**Male Social Status**

All animals were ‘chipped’ subcutaneously in the dorsal neck region (i.e. the scruff) with passive integrated transponder tags (PIT tags) (APT12, BioMark, Boise, ID, U.S.A.) 1 week before release into seminatural enclosures. PIT tags were implanted using a beveled needle (N125, BioMark) inserted into the scruff. Implantation without anaesthesia was performed quickly by experienced researchers to minimize handling time and stress on the mice. PIT tags are cylindrical, measuring 12.5 mm in length and 2.03 mm in diameter, and weighing 106 mg. At entry into seminatural enclosures, mice weighed an average of 17.04 g (range 10.06–34.52 g); thus, PIT tags as a percentage of body mass ranged from 0.31% to 1.05%, with an average of 0.62%. All mice were monitored after implantation and none showed changes in mobility or signs of discomfort (e.g. scratching/grooming the area).

PIT tag readers (FS2001-F-ISO, BioMark) were placed above the food source in each subsection of seminatural enclosures. Standard rodent chow pellets were available to gnaw at through 1 cm hardware cloth, to prevent caching and require periodic visits. With each food source visit, PIT tag ID, time and reader location data were streamed to a computer and recorded with data-logging software (Mininom, Culver City, CA, U.S.A.). A male’s social status was considered ‘dominant’ if his implanted PIT tag ID represented >80% of male PIT tag reads in any one territory (specifically before infection), achieved by excluding all other males from optimal territories (Fig. 1b). All other males were considered ‘nondominant’ and typically cohabitated in suboptimal territories with relatively few nesting females. Males began competing for optimal territories immediately upon release and stable territoriality was typically reached in less than 2 weeks. In this experiment, stable dominance was achieved in all optimal territories within 8 days. Males maintain dominance through direct physical aggression (e.g. grappling and chasing) and posturing (e.g. rearing up and tail rattling) towards invading males as well as indirectly by scent marking with major urinary proteins (MUPs) (Hadilovksa et al., 2015; Hurst & Beynon, 2004; Nelson, Cauceglia, et al., 2013; Nelson, Colson, et al., 2013). Under normal conditions, male–male bouts occurred numerous times per 24 h, mostly at night, for each dominant male (W. K. Potts, personal observation). Female mice do form complex social structures and were also chipped, but those social structures are more difficult to interpret with PIT tag data alone (Auclair et al., 2014; Ferrari et al., 2019; Manning et al., 1995).

**Friend Virus Complex (FVC)**

The mouse-specific retrovirus used in this study, FVC, is composed of two viral genomes, Friend murine leukemia virus (FMuLV) and spleen focus-forming virus (SFFV), which synergistically cause disease in mice (Clark & Mak, 1983; Friend, 1957). FMuLV is a replication-competent gammaretrovirus, while SFFV, a related virus, is replication incompetent (reviewed in Hasenkug & Chesebro, 1997). They must coinfect the same cell to be packaged into virus particles composed of MuLV-encoded structural proteins. Horizontal transmission of FVC has been reported in wild-derived mice and primarily transmits between males (Cornwall et al., 2021). Virus used for infections was originally sourced from a biologically cloned virus (bioclon), grown using mono-cell culture previously described (Kubinak et al., 2012). Viral stock for this experiment was produced as follows: bioclon was used to infect 42 mice (males and females) of an inbred strain of house mice (DBA/2, The Jackson Laboratory, Bar Harbor, ME, U.S.A.) for a unrelated experiment, and virus-laden supernatant from homogenized spleens was collected. This supernatant was then pooled and aliquoted to abate freeze–thaw-associated degradation. We used 100 μl of this pooled stock to infect each mouse by intraperitoneal (IP) injection at the study’s midpoint (Fig. 1a, day 13). The bioclon stock virus itself was not used for infections because such infections in wild-derived mice result in low virulence and variation (Cornwall et al., 2021). Passage through DBA/2 mice increases FVC replication and virulence due to the susceptibility of this strain, leading to rapid viral adaptation (Kubinak et al., 2013, 2015).

**FVC Virulence**

The replication of FVC leads to dysregulated proliferation and differentiation of erythroblasts, specifically due to binding of the erythropoietin receptor by the truncated ENV gene product (GP55) of SFFV (Zhang et al., 2006). These erythroblasts then proliferate and are sequestered by the spleen, leading to spleen enlargement (i.e. splenomegaly) and occasionally spleen rupture. As erythroblasts are the primary target cell for FMuLV, the uncontrolled proliferation of cells caused by SFFV increases the probability of obligate co-infection by both SFFV and FMuLV (Kosmider & Moreau-Gachelin, 2006; Ruscetti, 1999). Virulence was primarily quantified by measuring spleen mass of infected animals to the nearest 0.01 g. We also assessed spleen mass corrected for body mass (i.e. relative spleen mass), which yielded nearly identical results, but effect sizes that are reported as relative spleen mass (i.e. grams of spleen per gram of body) are more difficult to interpret. Because these animals had similar body masses, and there was low variation in uninfected spleen mass, we report absolute spleen mass here. A soft and variable upper limit on this measure of virulence is inherent due to an increased risk of spleen rupture as spleen mass increases, censoring infected spleen mass and proviral load for animals that died prior to day 12. However, we included these animals in the survivorship analysis.

**FVC Replication: Proviral Load of FMuLV and SFFV**

We estimated FMuLV replication by quantifying the proportion of retroviral genome integrations per host genome (i.e. proviral load) from DNA extracted from homogenized whole spleens of infected animals using a qPCR system previously described (Kubinak et al., 2012) on the BioRad CFX96 platform. Briefly, we used primers specific for the FMuLV genome to quantify proviral insertions relative to the mouse housekeeping gene GAPDH. Proviral load was favoured as our measure of viral replication because it represents a discrete completion of the retroviral lifecycle: transmission, adhesion, injection, migration, reverse transcription and finally integration. Although defective viral particles are capable of host–genome integration, there are relatively fewer than would be quantified via RNA assessments. Presence or absence of SFFV was determined using the qPCR system described above. A melt-curve peak of 75.0 °C indicates the presence of integrated SFFV provirus. However, SFFV could only be confidently assessed for infection status (positive/negative) because of the high level of similar endogenous DNA and expressed RNA in wild-derived mice, which amplify in competition with SFFV during qPCR (Cornwall et al., 2021).
Ethical Note

We carried out our experiments involving live animals in accordance with all applicable federal and state laws and institutional guidelines. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Utah under protocol no. 17-07021. Because our research questions involve the interaction of complex social behaviour and the effects of pathogen infection, we required the use of live animals in a social species. *Mus musculus* is an ideal model system for this investigation because of the wealth of knowledge available on the biology and social behaviour of this species, which ensures that we could gain the maximum amount of insight possible from our results. For this study specifically, we could not use standard inbred laboratory strains of mice, as they lack normal behaviours required for our seminatural enclosures (Nelson, Cauceglia, et al., 2013; Nelson, Colson, et al., 2013). Thus, we used mice from an outbred wild-derived colony that exhibit these necessary behaviours. Because these wild-derived mice are less well suited to living in captivity, we have modified the standard mouse care procedures to reduce the amount of disturbance experienced by mice in our colony. Specifically, cage changes are done every 2 weeks, rather than weekly, to reduce the handling time and minimize the amount of calcitriol, expensive scent marking that wild-derived mice exhibit. We sought to minimize the number of individuals used in this study in two ways: first, we conducted a pilot experiment comparing the effect of various housing conditions on FV virulence, specifically individually housed mice, mice paired in a standard breeding cage (1 male and 1 female) and mice housed in seminatural enclosures. The greatest variation in virulence was found among mice in seminatural enclosures, particularly between dominant and nondominant mice. This prompted us to continue experiments in seminatural enclosures comparing FV virulence in dominant versus nondominant males. Second, we used our experience with previous seminatural enclosure experiments to estimate how many enclosures would be required to generate enough dominant and nondominant males needed for our statistical analyses. A power analyses indicates that ~20 dominant males are required to detect a 20% effect, assuming the mean and variation in lyses. A power analyses indicates that ~20 dominant males are needed for our statistical analyses. Among mice in seminatural enclosures, particularly between males, is a necessary feature of our experimental design. Male mice compete for control of territories, and this includes physical confrontations (e.g. biting, grappling and chasing). This can cause injuries to the mice, and those that are found to be severely injured during daily inspections of the enclosures are humanely euthanized (see below). This was an uncommon occurrence.

Mice in seminatural enclosures were captured by hand by researchers at the midpoint and end of the experiment and placed in standard cages for up to 2 h to allow for experimental procedures. At the midpoint of the experiment, mice were infected with Friend virus complex (FV) via intraperitoneal (IP) injection and immediately returned to their enclosures. Infection with FV can cause some distress due to spleen enlargement, but severe distress only occurs in cases of spleen rupture, which can lead to death. Outward signs of health decline associated with pending spleen rupture include cachexia and immobility in mice, which is readily observable by researchers during daily inspections, and any mouse exhibiting such signs is immediately removed from the enclosure and humanely euthanized.

Euthanasia was performed using carbon dioxide asphyxiation, using a displacement rate of 50% of the chamber volume per minute, which is followed by cervical dislocation. These primary and secondary methods together ensure a rapid loss of consciousness, followed by the timely arrest of respiratory and brain function. These are among the preferred primary and secondary methods of euthanasia for small adult rodents by the University of Utah's IACUC per American Veterinary Medical Association (AVMA) guidelines (Leary et al., 2020). Sampling of spleens for assessment of FV virulence and replication requires mice to be euthanized at the end point of the experiment.

Statistical Methods

We assessed differences in spleen mass of FVC-infected males compared to uninfected males, and the correlation between spleen mass and FVC proviral load using simple linear regressions (lm). Spleen mass and proviral load of infected males were assessed as separate response variables using the best-supported (lowest AICc) linear mixed-effect regression (lmer) models from the ‘lme4’ package in R (Bates et al., 2015; R Core Team, 2016). The full model included the independent fixed effects of social status (two factors, dominant or nondominant), enclosure temperature (two factors, warm or cool) and their interaction (social status*enclosure temperature), along with random effects to control for relatedness between individuals (45 breeding cage identities) and cohabitation of competitors (six replicate enclosures). The full model predicting infected spleen mass was tiered for the lowest AICc with the three other models that varied random effect structure, while maintaining both fixed effects and their interaction. The interpretation of the other models was consistent. The full model predicting FMuLV proviral load was lowest by more than 2 AICc overall. Thus, infected spleen mass and proviral load effects were from the full model. We assessed risk of mortality between groups with the ‘survival’ package in R (Therneau & Mayo Foundation, 1999) using Fisher's
RESULTS

Spleens of males housed in seminatural conditions and infected with FVC weighed, on average (± SEM), 0.74 ± 0.18 g, while those of similarly housed uninfected males weighed about 1/12th that size, 0.06 ± 0.02 g (lm: spleen mass ~ FVC: \( T = -3.75, P = 0.001 \)). When mortality occurred post infection (15 of 58 males), it was most often a result of spleen rupture (confirmed for nine of 15 animals). In the uninfected enclosure, one nondominant male died on the last day of competition from wounding. Despite that limitation, the heaviest uninfected enclosure, one nondominant male died on the last day of competition from wounding. Despite that limitation, the heaviest

Two males died prior to infection and were censored from all analyses. Animals that did not complete a 12-day infection were censored from spleen mass and proviral load analysis. Three males that survived the 12-day infection were below the limit of detection for SFFV amplification.

Viral replication of FMuLV was positively correlated with spleen mass as measured by spleen mass of infected animals (lm: spleen mass ~ FMuLV proviral load: \( T = 2.50, P = 0.016; \) Fig. 2). Notably, the modest \( R^2 \) of the correlation (0.12) also highlights the need to better explain the observed variation in virulence.

We found that, under normal warm conditions, FVC-infected nondominant males had spleens that were an average of twice the size of spleens of FVC-infected dominant males.

Specifically, upon dissection after surviving a 12-day infection, mean (± SEM) spleen mass was 0.44 ± 0.18 g for dominant males (\( N = 10 \)) and 0.98 ± 0.13 g for nondominant males (\( N = 17 \) (lm: \( T = -2.00, P = 0.052 \)); thus, infected dominant and nondominant males housed in cool enclosures experienced similar virulence levels (Fig. 3). Virulence of FVC infection for dominant males was similar across temperature conditions (lm: \( T = 1.121, NS \)).

Regarding mortality in warm and cool enclosures combined (i.e. overall), nondominance was associated with a higher risk of mortality following infection (Fig. 4). In particular, 13 out of 41 nondominant males did not survive the full duration, compared to one out of 17 dominant males (Fisher’s exact test: two-tailed \( P = 0.046 \)). The best-supported ATF model suggested that nondominant males survived 65% as long as dominant males following FVC infection (ATF: \( T = -1.95, P = 0.051 \)).

Dominant males in warm enclosures had significantly lower FMuLV replication than similarly housed nondominant males (lm: \( P = 0.016 \)) and significantly lower FMuLV replication than dominant males in cool enclosures (lm: \( P = 0.035 \)), while the interaction effect tended to be lower for nondominant males in cool enclosures (lm: cool enclosure-nondominant status: \( P = 0.091 \); Fig. 5, Appendix, Fig. A1). This reveals a relative increase in viral

Figure 2. Relationship between virulence of Friend virus complex (FVC), as measured by spleen mass of 12-day infected males, and proviral load of Friend murine leukemia virus (FMuLV). Points are infected males; the solid grey line is the overall regression.
replication efficiency and/or an inability of hosts to slow viral replication associated with nondominance and cool conditions. The SFFV component of FVC was detected in all but three males that survived the 12-day infection. Of the males with no detectable SFFV, one was dominant and two were nondominant. All SFFV-negative males were in different enclosures, one of which (a nondominant male) was in a cool enclosure and the others were in warm enclosures.

**DISCUSSION**

We hypothesized that dominant mice would have less severe FVC infections, both in terms of virulence as well as viral reproduction. Our results show that dominant male mice have significantly less virulent infections as measured by spleen mass and mortality of infected animals compared to nondominant males. Despite censoring due to spleen ruptures when measuring FVC virulence as spleen size, the biased mortality pattern lends added confidence to the differential virulence results. This difference in virulence was associated with reduced viral reproduction of FMuLV in dominant animals. We found no association of SFFV presence with social status, as only three males, divided between both social status conditions, lacked detectable SFFV by qPCR.

Our results support the possibility that high-status (i.e. dominant) animals mount a stronger antiviral immune response than low-status animals, consistent with findings in rhesus macaques (Snyder-Mackler et al., 2016). In seminatural enclosure systems, males maintain dominance through both direct physical aggression and posturing towards invading males and indirect competition by heavily investing in calorically expensive scent marking, with an expanded profile of major urinary proteins (Hurst & Beynon, 2004; Nelson, Cauceglia, et al., 2013; Nelson, Colson, et al., 2013). Because direct and indirect systems of aggression are thought to be associated with differing levels of chronic stress for lower-status individuals (Sapolsky, 2005), experimentally modifying the relative importance and frequency of each type of aggression in a species that uses both could be enlightening.

Differences in disease and health due to position and transition on the social landscape are thought to be largely mediated by steroid hormone regulation, most notably the glucocorticoid stress response (Sapolsky, 2005). High glucocorticoid levels can be adaptive during acute stress by diverting resources away from long-term functions to maximize an animal's ability to respond to the immediate stressor (Sapolsky, 2004). However, in prolonged or frequently repeated stress conditions (i.e. chronic stress), glucocorticoid-associated regulatory changes can lead to plausibly nonadaptive immunosuppression and other deleterious effects (Sapolsky, 2004, 2005), although this effect may be limited to humans and captive or domesticated animals (Beehner & Bergman, 2017). The relative level of chronic stress can be higher in either low- or high-status individuals depending on how social status is established and maintained. These differential outcomes can depend on species, sex (Lea et al., 2018) and possibly other environmental conditions as postulated here. Regulation of sex steroids, especially testosterone, has also been implicated in social status and social transitions (Schradin et al., 2009; Terburg & Van Honk, 2013), which are also influential in immune system regulation (Trigunaite et al., 2015).

During this study, the temperature of two replicate enclosures, experienced substantially lower temperatures than the other four replicates. This presented us with an opportunity to assess the effect of temperature, if any, on FVC virulence and replication. We found that temperature did associate with differential FVC infection outcomes, albeit in an unexpected and largely mysterious way. Cool enclosures appeared to abolish the pattern of higher virulence in nondominant males observed in warm enclosures, with infected males in cooled enclosures having similar spleen mass regardless of social status. This comparison could plausibly be confounded by variable spleen enlargement limits, exemplified by the heaviest
infected spleen observed in cool enclosures being 83% the size of the largest in warm enclosures (1.85 g compared to 2.24 g in males of similar body mass). However, there were no significant differences in mortality between warm and cool enclosures, nor any relative change in mortality of nondominant males compared to dominant males (Fig. 4). For dominant males, cooled enclosures were also associated with an increase in FeLV proviral load (Fig. 5). More experiments are required to verify whether the interaction effect of temperature abolished the pattern of status-dependent infection severity, or in fact, reversed it. The complex interaction observed between social status and enclosure temperature is difficult to interpret with the current data from this unplanned manipulation.

Changes in temperature could directly affect pathogen virulence, as seen in some in vitro studies of rhinovirus infection in mouse airway cells (Foxman et al., 2015) and/or indirectly by changing the importance and intensity of competition in mice (Welden & Slauson, 1986). Cooler environments may lower the intensity of competition (i.e. the strain induced by competition) by increasing the relative importance of other sources of strain, such as thermo-regulation and nest maintenance (Phifer-Rixey & Nachman, 2015). Thus, changes in temperature may impact behaviour and endocrine changes associated with social status and, in turn, indirectly influence the associated disease susceptibility. Future experiments that manipulate and monitor social status in seminatural enclosures must ensure precise and frequent temperature monitoring and direct observations of male social behaviours (e.g. bout frequency and severity and time spend patrolling territories) (Meagher et al., 2000; Potts et al., 1991). Although intentional manipulation of enclosure temperature in an HVAC-controlled facility is difficult, especially when simultaneous warm controls need to be maintained, further exploration is worthy of pursuit.

Our findings further demonstrate that health differences associated with social status extend to infectious disease and that mitigating these effects through modifying environmental context is plausibly effective. We show that modifying environmental factors, such as temperature, may be profoundly useful in understanding the factors that impact virulence, and that associations of social status and virulence, even within one sex of a single species, may be context dependent. This emphasizes the importance of considering abiotic environmental factors in combination with biotic influences on pathogen virulence and overall health. A more integrated understanding of these factors, and how they interact with each other, is necessary for improving models of virulence and virulence evolution. This exciting — yet unexpected — discovery provides new potential methods for manipulating the intensity of competition and social conflict in the most economic and well-studied mammalian model available to science.

One must remember that our data showed correlations between social status and pathogen virulence. Consequently, causation is impossible to determine. Social status could cause or simply predict differences in susceptibility to FVC linked with accrued or predisposed status-associated physiological attributes (e.g. wounding or genetics). It has been previously demonstrated that social status can be experimentally manipulated by using several rounds of monitored competition and a ‘winners—losers’ bracket design, where like-status males compete with novel competitors after status is initially established (Cauceglia et al., 2020). This design ensures that some previously nondominant animals become dominant, and vice versa, while an unpredictable subset maintains their previous status. Ideally, infection severity would be measured before and after social status transitions in the same individual and compared to when status is maintained. Unfortunately, FVC infections frequently become chronic and, even when cleared, can result in experimental design complications associated with adaptive immunity, namely immunological memory, which make repeat measures of virulence at times of different social status unavailable. Other experimental designs in this system, utilizing other pathogens, do allow necessary repeated measures of virulence from the same individual through time (e.g. malaria and its associated anemia; Lin et al., 2017) and after changes in social status (Cauceglia et al., 2020).

Moving forward, we can now conduct powerful experiments that could causally link social status and infectious disease severity in mice and investigate the mechanistic underpinnings of such effects more broadly. It will be important in future studies to link infection severity, or in fact, reversed it. The complex interaction observed between social status and enclosure temperature is difficult to interpret with the current data from this unplanned manipulation.

Changes in temperature could directly affect pathogen virulence, as seen in some in vitro studies of rhinovirus infection in mouse airway cells (Foxman et al., 2015) and/or indirectly by changing the importance and intensity of competition in mice (Welden & Slauson, 1986). Cooler environments may lower the intensity of competition (i.e. the strain induced by competition) by increasing the relative importance of other sources of strain, such as thermo-regulation and nest maintenance (Phifer-Rixey & Nachman, 2015). Thus, changes in temperature may impact behaviour and endocrine changes associated with social status and, in turn, indirectly influence the associated disease susceptibility. Future experiments that manipulate and monitor social status in seminatural enclosures must ensure precise and frequent temperature monitoring and direct observations of male social behaviours (e.g. bout frequency and severity and time spend patrolling territories) (Meagher et al., 2000; Potts et al., 1991). Although intentional manipulation of enclosure temperature in an HVAC-controlled facility is difficult, especially when simultaneous warm controls need to be maintained, further exploration is worthy of pursuit.

Our findings further demonstrate that health differences associated with social status extend to infectious disease and that mitigating these effects through modifying environmental context is plausibly effective. We show that modifying environmental factors, such as temperature, may be profoundly useful in understanding the factors that impact virulence, and that associations of social status and virulence, even within one sex of a single species, may be context dependent. This emphasizes the importance of considering abiotic environmental factors in combination with biotic influences on pathogen virulence and overall health. A more integrated understanding of these factors, and how they interact with each other, is necessary for improving models of virulence and virulence evolution. This exciting — yet unexpected — discovery provides new potential methods for manipulating the intensity of competition and social conflict in the most economic and well-studied mammalian model available to science.

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outcomes to host immune system regulation to build mechanistic models of how social status and the environmental context affect virulence. Such studies should examine immunological variables such as leukocyte populations, cytokine expression and antibody levels during infection. Measures of glucocorticoid levels such as cortisol in the context of infection could also provide mechanistic insight into how such immunological differences are established in the first place. Moreover, new methods of manipulating environmental context (e.g. temperature) to plausibly modify the intensity and importance of competition and perceived social differences create new possibilities for investigating the mechanistic underpinnings of health and disease disparities associated with more and less equal social environments (Pickett & Wilkinson, 2015; Sapolsky, 2004; Welden & Slauson, 1986). Future investigations that include more robust behavioural monitoring of competition and the associated endocrinology and immunology are being proposed to validate this system of environmental context manipulation. We aim to help determine the causative factors underlying the correlation between subjective social status and susceptibility to infection observed in this study and in many human societies.

Data Availability

Any persons interested in using the data associated with this manuscript are welcome to access our data deposit (Dryad: https://doi.org/10.5061/dryad.th76hdxs). To better understand and accurately utilize these data, we welcome anyone interested to contact the co-first authors. The deposited data set includes all the data and descriptions for used or excluded factors in the infected male analysis. https://datadryad.org/stash/share/DaTp83ewf3mQGqWH7DpsMNmw501uNnWpv9p3RqpqnY.

Author Contributions

Derek L. Stark: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Visualization; Roles/ Writing — original draft; Writing — review & editing. Joseph W. Cauceglia: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Visualization; Roles/Writing — original draft; Writing — review & editing. Victoria N. Sitzman: Investigation; Methodology; Writing — review & editing. Mayra C. Repetto: Investigation; Methodology; Writing — review & editing. Jacob M. Tadje: Investigation; Methodology; Writing — review & editing. Wayne K. Potts: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing — review & editing.

Conflicts of Interest

None.

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## Appendix

![Figure A1](image-url) Effect size estimates for each factor and their interaction from models predicting (a, b) spleen mass and (c, d) proviral load when levelled (zeroed) on (a, c) dominant males in cool enclosures and (b, d) dominant males in warm enclosures. *P < 0.05