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Urinary volatile compounds differ across reproductive phenotypes and following aggression in male Siberian hamsters

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HIGHLIGHTS

- Siberian hamsters use urine to scent mark their territories.
- Male hamsters displaye a diverse composition of urinary compounds.
- Long-day reproductive males have high levels of individual pyrazines and ketones.
- Aggression predominately alters methyl pyrazines in long days.
- Aggression, testosterone and urinary compounds are associated in long days.

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ABSTRACT

Chemical communication plays an integral role in social behavior by facilitating social encounters, allowing for the evaluation of social partners, defining territories and advertising information such as species and sex. Odors provide information about the social environment for rodents and other mammals; however, studies identifying chemical compounds and their functions have thus far focused primarily on a few species. In addition, considerably less attention has been focused on how environmental factors and behavioral context alter these compounds during periods of reproductive quiescence. We examined the effects of photoperiod and social context on chemical communication in the seasonally breeding Siberian hamster which displays modest territorial aggression during long "summer-like" days, but *increased* aggression in short "winter-like" days. We collected urine samples from long- and short-day male hamsters to investigate how photoperiod and subsequent changes in reproductive phenotype alter urinary volatile compound profiles. Next, we identified changes in urinary compounds before and after an aggressive encounter. Male hamsters exhibited a diverse urinary profile across photoperiods; however, long-day reproductive males showed higher levels of individual compounds when compared to short-day non-reproductive males. In addition, individual compounds were altered following an aggressive encounter; some changed only in long days whereas others changed regardless of photoperiod. Further, aggression and circulating levels of testosterone were positively correlated with urinary compounds in long-, but not short-day males. These findings suggest both photoperiod- and aggression-specific physiological regulation of urinary compounds in this species and contribute to a greater understanding of chemical communication more broadly.

1. Introduction

Chemical communication is a key aspect of social behavior in virtually all animals. These communicative signals are used to facilitate social encounters, evaluate social partners, establish territories and advertise information such as identity and sex [1,2]. Various types of communication, including visual, auditory, tactile, or chemical signals, can be transmitted between sender and receiver. Such modes of communication have been studied across many vertebrate taxa, however rodents offer an excellent comparative model to study mammalian chemical communication [2,3]. Rodents are differentiated from other vertebrate taxa because sense of smell is the most important sensory modality; therefore, olfaction provides a sensory map of the environment [4]. In fact, there are functional consequences for rodents with compromised olfaction. For example, mice with disrupted olfaction display abnormal aggressive [5–7] and sexual behaviors [7]. Further, distinct chemical compounds that induce aggression [8], dominance [9] and reproductive partner preference [10] have been examined in mice. Identifying

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functional behavioral consequences and attributing individual compounds to such behaviors is key to a comprehensive understanding of mammalian chemical communication.

The importance of olfaction is clear from evidence in olfactory deficient mice and behavioral responsivity to individual chemical compounds [5–10]; however, these findings have not been extended to mammalian chemical communication more broadly. Instead, work in mammals has increasingly focused on a relatively limited number of species, particularly mice (but see [11–15] for some notable exceptions). In addition, much of the work on the chemical complexity in rodents has focused on animals that are reproductively functional or on behaviors expressed within a reproductive context. For example, the role of the major histocompatibility complex (MHC) in the production of chemical compounds, and the functions of these compounds, has been examined predominantly within mate choice paradigms [2–4]. Further, the use of kin recognition and the functions of scent marking have typically been studied within a reproductive context [24,16,17]. Despite these advances in rodent chemical communication much less is known about how changes in reproductive phenotype, and subsequent changes in endocrine status, within a non-reproductive social context influence individual chemical compounds. Using ecologically relevant model systems to study how the environment, including social and non-social factors and endocrine status, influence chemical signaling strongly expands upon the important findings that have emerged from the mouse model system.

Siberian hamsters (*Phodopus sungorus*) breed seasonally and are well established for the study of photoperiodic changes in physiology and behavior [18,19], making this species an excellent model to examine how season and behavioral context alter individual urinary compounds. First, hamsters in short "winter-like" days exhibit gonadal regression and display *increased* territorial aggression when compared with hamsters in long "summer-like" days [18,20]. This inverse relationship between gonadal steroids and aggression makes this species amenable to disassociating the individual effects of reproductive phenotype and aggression on chemical signaling. Second, Siberian hamsters employ chemical signaling in a variety of ways, including the use of urine, feces, sacculi and ventral glands in the wild and in the laboratory [21–24].

In the wild, males deposit their scent marks, composed of both urinary and ventral gland secretions, on the boundaries and throughout their home ranges [24]. The location of these chemical signals suggests that males use urinary and ventral gland signals for territorial defense [24]. In further support of this, sexually experienced males scent mark over conspecific male urine in a laboratory setting, therefore urine seems to be a signal involved in scent marking duels [21]. In addition to urinary signaling, male and female hamsters actively excrete ventral gland compounds across breeding seasons [25]; for males, they are differentially altered by photoperiod and aggression [25]. Males in short days generally display elevated levels of ventral gland compounds and further show aggression-induced elevations [25]. Moreover, male hamsters presented with ventral gland secretions display increased investigation of the secretion in short days when compared with long days [26]. These data suggest that ventral gland signaling is used more during periods of reproductive quiescence [25], whereas urinary signaling may be used more during periods of reproductive activity [21]. During the breeding season, hamsters survive with little rainfall therefore produce highly concentrated urine [27,28]. This corroborates high concentrations of urinary compounds that have previously been reported in laboratory derived male hamsters; females have low levels when compared to males [29]. These data suggest that concentrated urine, an adaptation for surviving in harsh Siberian summers, may be a chemical signal used more during the long days of the reproductive breeding season than in the short days of the non-breeding season [21,25]. By identifying specific urinary compounds that change with aggression and reproductive phenotype (*i.e.*, breeding condition and levels of the gonadal hormone testosterone), we can begin to connect the complexity of odors in Siberian hamsters to their functional significance.

In this study, we examined photoperiodic changes in the urinary compound composition of male Siberian hamsters, as well as aggression-induced changes of individual urinary compounds. We hypothesized that photoperiod and aggression would induce changes in individual urinary compounds. We also investigated the potential role for photoperiodic variation in the gonadal androgen testosterone (T) to regulate urinary compound composition. It is known that short-day males with regressed gonads display increased aggression despite low levels of T, whereas males in long days display less aggression concomitant with elevated T levels [18]. Therefore, we predicted that specific urinary compounds would be differentially associated with T concentrations across photoperiods. To test this, we collected urine and identified and quantified urinary volatile organic compounds (VOCs) using gas chromatography-mass spectrometry (GC-MS) [29]. Urine samples were collected from individual hamsters across photoperiods and prior to and following an aggressive encounter. The findings from this study allowed us to identify photoperiod- and behavior-specific changes in urinary compounds and further provide an understanding of the chemical ecology of mammalian chemical communication within an aggressive context.

2. Materials and methods

2.1 Animal housing and photoperiodic treatment

Adult (>60 days of age) hamsters were reared in a breeding colony maintained at Indiana University, Bloomington. Hamsters were bred and subsequently housed under long days (light:dark, 16:8 h) and group-housed at weaning (postnatal day 18). Ambient temperature was maintained at 20 ± 2 °C and relative humidity at 55 ± 5%. Hamsters were given *ad libitum* access to laboratory rodent chow (Lab Diet 5001, PMI Nutrition) and water. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee at Indiana University.

Resident hamsters were individually housed ($n = 32$) and intruder hamsters were group housed (4 animals per cage; males, $n = 16$) in the colony room for a one-week acclimation period. Subsequently, a subset of hamsters was transferred to a room on a short-day light cycle (light:dark: 8:16 h), whereas the remaining hamsters were relocated to a new room on the long-day light cycle (light:dark, 16:8 h). All hamsters remained in their photoperiodic regime for eight weeks. Reproductive state was determined based on *a priori* criteria previously established for Siberian hamsters [18]. Hamsters were characterized as reproductively competent if they had functional reproductive testes, displayed no significant change in body mass (<10%) and maintained a brown/grey coat color (long days; $n = 12$). Reproductive incompetence, in contrast, was characterized by regressed testes, a significant decrease in body mass (>10%) and a white coat color (short days; $n = 16$). A subset of animals did not respond, with respect to reproductive physiology, to the short-day photoperiodic regime based on our *a priori* criteria [18]. This reproductive morph that persists in short days has been previously documented [30,31]. These animals (short-day non-responders: $n = 4$) were excluded from further analyses due to insufficient numbers.

2.2 Aggressive behavior recording and analysis

Same-sex aggressive encounters were recorded and analyzed using previously outlined methods [18,20,32]. Aggression was assessed using the resident-intruder paradigm, which consists of placing an unfamiliar intruder hamster into the home cage of a resident hamster and allowing them to interact for 5 min, within the first 2 h of the dark phase. Hamsters were approximately the same age and mass (±10%) and from different parents. All trials were recorded under low illumination red lights using a Sony HandyCam Digital Camcorder HDR-SR7. Trained observers used

ODLog™ (Macropod Software, Eden Prarie, MN) to score aggressive behaviors (*i.e.*, attacks, chases and latency to first attack). Scores from two individuals were averaged and inter-rater reliability was accepted if <10% variation occurred. A principal components analysis (PCA) was used on these aggression variables to generate a composite score for each hamster. Aggression variables loaded strongly onto the first component and explained 76.16% of the total variance (Table S1); therefore we used PC1 as an aggression score for subsequent analyses.

2.3 Identification and quantification of urinary volatile compounds

In order to identify and quantify hamster urinary compounds, male urine was collected 1 h before behavioral trials (week 8, pre-aggression samples) and immediately following (<5 min; week 8, post-aggression samples) behavioral trials. The changes in urinary volatiles from pre- to post-aggression were likely not due to *de novo* synthesis/metabolism, but rather were volatiles stored in the bladder and differentially excreted in the absence (pre-aggression) or presence (post-aggression) of a social encounter (see examples of compounds added to urine upon elimination: [8,9,33]; reviewed in: [34,35]). An additional subset of animals were sampled prior (week 0, baseline; $n = 14$) and following photoperiodic treatment (week 8, pre-aggression; $n = 14$), in order to quantify repeatability of urinary compounds within individuals and to gauge changes in urinary compounds that occur in the absence of a social encounter (i.e., reproducibility). Individual male hamsters were placed in a clean cage and urine was collected from the bottom of the cage as soon as the animal urinated. All urine samples were collected during the dark phase to control for potential circadian fluctuations. Samples were frozen at −20 °C until analyzed and were thawed just prior to the sample preparation. If the sample was contaminated (*e.g.*, feces), it did not undergo chemical analysis.

Urinary VOCs were identified and quantified using gas chromatography-mass spectrometry (GC-MS) using previously established protocols [29]. Volatile compounds were extracted from 0.5 mL of undiluted urine using the stir bar sorptive extraction method (Twister™, 10 mm, 0.5 mm film thickness, 24 μL polydimethylsiloxane (PDMS) volume) for 60 min. Stir bars were obtained from Gerstel GmbH (Mülheim an der Ruhr, Germany) and were conditioned in the TC-2 tube conditioner (Gerstel GmbH) at 290 °C under high helium flow. An internal standard, 8 ng of 7-tridecanone (Aldrich, Milwaukee, WI), was added in 5 μL of methanol (J.T. Baker, Avantor Materials, Phillipsburg, NJ) to pre-cleaned 20 mL glass scintillation vials containing 2.0 mL high-purity water (OmniSolv™, EM Science, Gibbstown, NJ), followed by the addition of a preconditioned stir bar. Stirring speed was 850+ rpm on the Variomag Multipoint HP 15 stirplate (H + P Labortechniq, Oberschleissheim, Germany). Prior to extraction, all glassware was washed with acetone and dried in the oven at 80 °C. After extraction, the stir bars were placed into the TDSA desorption tubes in the autosampler.

Quantitative analysis was performed using the Agilent 6890N gas chromatograph connected to a 5973i MSD mass spectrometer (Agilent Technologies, Inc., Wilmington, DE) with the thermal desorption autosampler and cooled injection system (Gerstel TDSA-CIS-4). Positive electron ionization mode at 70 eV was used with a scanning rate of 2.47 scans/s over the mass range of 41–350 amu. The mass spectrometric detector (MSD) transfer line temperature was set at 280 °C. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. The separation capillary was DB-5MS (30 m × 0.25 mm, inner diameter [i.d.], 0.25 μm film thickness) from J&W Scientific, Folsom, CA. Samples were thermally desorbed in a Thermal Desorption Autosampler (TDSA), followed by injection into the column with a cooled injection assembly (CIS-4). The TDSA operated in a splitless mode. The temperature program for desorption was 20 °C (0.5 min hold time), then 60 °C/min to 280 °C (10 min hold time). Temperature of the transfer line was set at 280 °C. CIS was cooled with liquid nitrogen to −80 °C. After desorption and cryotrapping, CIS was heated at 12 °C/s to 280 °C (10 min hold time). CIS inlet was operated in the solvent vent mode, a vent pressure of 9 psi, a vent flow of 30 mL/min, and a purge flow of 50 mL/min. The temperature program in the GC operation was 40 °C for 5 min, then increasing to 200 °C at the rate of 2 °C/min (10 min hold time). The carrier gas head pressure was 9 psi (flow rate, 1.1 mL/min at the constant flow mode).

2.4 Quantification of urinary volatile compounds

Peak areas of the identified compounds were used for quantitative comparisons between long days and short days (Table 1). Identifications were based on the comparisons of the retention times and spectra of the known standard compounds. Peak areas of each compound were normalized by dividing each peak area by that of the area of the internal standard in the same analytical run. Peak areas with a mean relative proportion of <0.1% were identified, but were not included in further statistical analyses.

Table 1
List of identified urinary compounds from male hamsters.

Compounds	Retention time (min)
Acids	
Benzoic acid	30.07
Hexadecanoic acid	76.42
Aldehydes	
Phenylacetaldehyde	19.86
Nonanal	24.50
Decanal[a]	31.68
Alkenols	
Trans-2-octen-1-ol	22.64
Amides	
N-phenylformamide	31.98
Hexadecanoamide	83.99
9-Octadecanoamide	93.99
Aromatic amines	
o-Toluidine	21.63
Esters	
Ethyl benzoate	29.02
Ethyl salicylate	36.36
Propyl benzoate	36.60
Heterocyclics and aromatics	
2,6-Dimethylpyridine[a]	9.27
2,5-Dimethylpyrazine[a]	11.15
2-Acetyl-1-pyrroline	12.29
3-Hepten-2-one[a]	12.98
A trimethylpyrazine[a]	16.92
A propylpyrazine[a]	17.37
2-Methylvinylpyrazine	18.10
Acetophenone[a]	21.30
2-Methyl-5-propylpyrazine	23.50
An ethylvinylpyrazine	23.62
A C3-vinylpyrazine	29.91
A C4-vinylpyrazine	35.16
A dipropylpyrazine	35.97
Indole[a]	37.20
Ketones and lactones	
4-Heptanone[a]	8.97
2-Heptanone[a]	9.96
6-Methyl-5-hepten-2-one[a]	15.95
3-Octen-2-one	20.38
4-Nonanone[a]	22.09
γ-6-Dodecalactone	60.08
Terpenes	
Limonene	18.88
p-Cymene	18.90
o-Cymene	19.24
m-Cymene	19.90
Perillene	24.66
Geranylacetone	47.40

[a] Urinary compounds also identified in a previous study (Soini et al., [29]).

2.5 Blood sampling and quantification of testosterone

After eight weeks of the photoperiodic regime, blood samples were drawn from the retro-orbital sinus 24 h prior to behavioral trials (*i.e.*, pre-aggression) and then again within 5 min of completion of behavioral trials (*i.e.*, post-aggression). Serum testosterone (T) was quantified using an enzyme immunoassay (EIA; Assay Design 900–065; assay sensitivity = 5.67 pg/mL) that has been validated in this species and has negligible or undetectable cross-reactivity with other steroid hormones [32]. Samples were diluted (1:20 or 1:40), assayed in duplicate according to the manufacturer's recommended protocol and were balanced across two plates of the same kit lot. Samples with C.V. >10% and maximum binding <20% or >80% were re-analyzed. Intra-assay variability was <4.5% and inter-assay variability was 2.98%.

2.6 Statistical analyses

All statistical analyses were run in JMP v. 11.0.0 (SAS Institute, Inc., Cary, NC) and statistical significance was attributed at $p < 0.05$. We transformed each variable to attain normality and equal variances. Principal component analyses (PCAs) were conducted on aggression data (Table S1) as well as baseline, pre- and post aggression levels of urinary compounds (Table 2 and Table S2). PCAs were used to reduce the dimensionality of the data by loading inter-correlated variables into independent components as well as to determine how urinary compounds group together; variables that loaded strongly (≤−0.5 or ≥0.5) within a component confirm statistical relationships between those compounds. PCs with an eigenvalue >1 were retained for analysis. Paired *t*-tests were used to compare baseline (week 0) and pre-aggression (week 8) levels of urinary compounds within groups to determine reproducibility of compounds. In addition, repeatability of urinary compounds of each individual was calculated on PCs generated for urinary compounds as described previously [36]. T-tests were used to compare reproductive physiology, aggression measures, pre-aggression levels of urinary compounds and T between long- and short-day animals. Repeated-measures ANOVAs were used to compare pre- and post-aggression urinary compounds and T levels, followed by paired *t*-tests if there was a significant photoperiod x aggression interaction. Spearman's rank correlations were used to quantitatively assess relationships between aggression, T levels (both pre- and post-aggression values) and PCs generated for pre-and post-aggression urinary compounds.

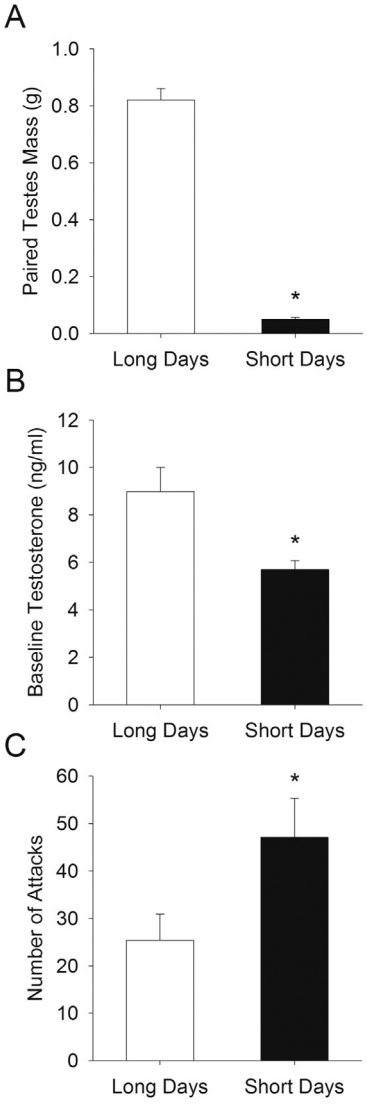

Fig. 1. Photoperiodic changes in aggression and reproductive phenotype. (A) number of attacks; (B) paired testes mass; (C) baseline testosterone. Bar heights represent means ± S.E.M. *Statistically significantly different (*t*-test, $p < 0.05$).

3. Results

3.1 Short-day hamsters had non-functional reproductive physiology but were more aggressive than long-day hamsters

Short-day males had significantly decreased body mass (16%, $t_{26} = -6.43$, $p < 0.001$), regressed testes mass ($t_{26} = 20.09$, $p < 0.001$; Fig. 1A) and decreased circulating levels of T ($t_{26} = 4.34$, $p = 0.001$; Fig. 1B), when compared with long-day males. Short-day males also displayed more ($t_{26} = 2.19$, $p = 0.03$; Fig. 1C) and longer ($t_{26} = 3.89$, $p = 0.03$) attacks, more ($t_{26} = 3.11$, $p = 0.04$) and longer ($t_{26} = 3.39$, $p = 0.04$) chases and displayed more overall aggression (aggression score: $t_{26} = 3.80$, $p = 0.04$; Table S1). The latency to first attack did not differ across photoperiods ($t_{26} = -1.08$, $p = 0.15$).

3.2 Siberian hamsters displayed a diverse and repeatable composition of urinary compounds

Through analyzing individual GC-MS generated total ion chromatograms (TIC) (Fig. S1) we identified 39 urinary VOCs from several compound classes: aldehydes, alkenols, amides, amines, esters, heterocyclics and aromatics, ketones and terpenes (Table 1). Among the 39 identified compounds, we quantitatively compared 24, which were compounds that had a relative proportion >0.1%.

Urinary VOCs from baseline (week 0) to pre-aggression (week 8) did not statistically differ across sampling timepoints, therefore, were highly reproducible (PC1: $t_{13} = -2.26$, $p = 0.77$; PC2: $t_{13} = 1.44$, $p = 0.72$; Table S2) and repeatable (PC1: $F = 1.58$, $r = 0.69$, $p = 0.007$; PC2: $F = 2.10$, $r = 0.52$, $p = 0.02$; Table S2). Further, when examining each specific VOC, all 24 were highly reproducible within individuals when maintained in long days (p's > 0.05; Supplementary Material – Results). These results indicate that urinary VOCs are reliable within individuals and consistently different among individuals.

3.3 Reproductive phenotype altered urinary compound levels

Male urinary compound compositions did not change across photoperiods; all urinary compounds were present in all hamsters. However, long-day males generally had elevated levels of each compound. Ten urinary compounds were elevated in long days when compared with short days, including five pyrazines and two ketones: *N*-phenylformamide ($t_{26} = -2.67$, $p = 0.001$); 2-acetyl-1-pyrroline ($t_{26} = -1.96$, $p = 0.04$); a propylpyrazine ($t_{26} = -1.82$, $p = 0.04$); 2-methylvinylpyrazine ($t_{26} = -2.55$, $p = 0.002$); 2-methyl-5-propylpyrazine ($t_{26} = -2.69$ $p = 0.02$); an ethylvinylpyrazine ($t_{26} = -2.86$, $p = 0.03$); a C4-vinylpyrazine ($t_{26} = -2.42$, $p = 0.04$);

γ-6-dodecalactone ($t_{26} = -2.11$, $p = 0.02$); 4-heptanone ($t_{26} = -3.88$, $p = 0.04$); and 2-heptanone ($t_{26} = -1.82$, $p = 0.03$). In contrast, only one trimethylpyrazine compound was elevated in short days when compared with long days: a trimethylpyrazine ($t_{26} = 1.52$, $p = 0.03$) (Table S3).

Thirteen urinary compound levels did not change with photoperiod, including two 2,5-dimethylpyrazines: phenylacetaldehyde ($t_{26} = -1.60$, $p = 0.56$); nonanal ($t_{26} = 1.06$, $p = 0.47$); decanal ($t_{26} = -1.59$, $p = 0.56$); *o*-toluidine ($t_{26} = -0.99$, $p = 0.34$); hexadecanoic acid ($t_{26} = -1.51$, $p = 0.62$); ethyl benzoate ($t_{26} = 1.91$, $p = 0.18$); 2.5-dimethylpyrazine ($t_{26} = 0.09$, $p = 0.50$); acetophenone ($t_{26} = -1.60$, $p = 0.56$); a C3-vinylpyrazine ($t_{26} = 1.98$, $p = 0.34$); indole ($t_{26} = 1.76$, $p = 0.23$); 6-methyl-5-hepten-2-one ($t_{26} = 0.23$, $p = 0.82$); 4-nonanone ($t_{26} = -0.68$, $p = 0.51$); and limonene ($t_{26} = -0.43$, $p = 0.68$) (Table S3).

3.4 Aggression altered urinary compound levels

Nine urinary compounds increased following an aggressive encounter in long-day males, but not short-day males: *N*-phenylformamide ($F_{1,26} = 6.74$, $p = 0.02$); 2-acetyl-1-pyrroline ($F_{1,26} = 5.13$, $p = 0.03$); a trimethylpyrazine ($F_{1,26} = 3.47$, $p = 0.03$); a propylpyrazine ($F_{1,26} = 12.08$, $p = 0.02$); 2-methylvinylpyrazine ($F_{1,26} = 12.21$, $p = 0.002$); 2-methyl-5-propylpyrazine ($F_{1,26} = 2.77$, $p = 0.04$); a C3-vinylpyrazine ($F_{1,26} = 3.67$, $p = 0.02$); γ-6-dodecalactone ($F_{1,26} = 3.61$, $p = 0.04$); and 6-methyl-5-hepten-2-one ($F_{1,26} = 2.22$, $p = 0.04$) (Fig. 2). An additional six urinary compounds increased following an aggressive encounter regardless of photoperiod, including one aldehyde, two pyrazines and three ketones: nonanal ($F_{1,26} = 2.57$, $p = 0.02$); 2,5-dimethylpyrazine ($F_{1,26} = 3.21$, $p = 0.04$); an ethylvinylpyrazine ($F_{1,26} = 6.12$, $p = 0.02$); 4-heptanone ($F_{1,26} = 2.73$, $p = 0.01$); 2-heptanone ($F_{1,26} = 2.05$, $p = 0.02$); and 4-nonanone ($F_{1,26} = 3.50$, $p = 0.04$) (Fig. 3).

Nine urinary compounds did not change following an aggressive encounter: phenylacetaldehyde ($F_{1,26} = 0.51$, $p = 0.48$); decanal ($F_{1,26} = 0.33$, $p = 0.57$); *o*-toluidine ($F_{1,26} = 0.76$, $p = 0.39$); hexadecanoic acid ($F_{1,26} = 0.02$, $p = 0.89$); ethyl benzoate ($F_{1,26} = 0.42$, $p = 0.53$); acetophenone ($F_{1,26} = 2.02$, $p = 0.17$); a C4-vinylpyrazine ($F_{1,26} = 1.46$, $p = 0.24$); indole ($F_{1,26} = 2.02$, $p = 0.17$); and limonene ($F_{1,26} = 1.68$, $p = 0.21$) (Fig. 4).

3.5 Urinary compounds were associated with aggression and T

Two PCAs were conducted on pre-aggression and post-aggression urinary compounds to determine how urinary compounds group together; variables that loaded strongly (≤−0.5 or ≥0.5) within a component confirmed statistical relationships between those compounds (Table 2). Two pre-aggression urinary compound PCs were extracted ($PC1_{PRE\text{-}AGG\ COMPOUNDS}$ and $PC2_{PRE\text{-}AGG\ COMPOUNDS}$) which explained 49.45% of the total variance (Table 2). For post-aggression urinary compounds, two PCs were extracted ($PC1_{POST\text{-}AGG\ COMPOUNDS}$ and $PC2_{POST\text{-}AGG\ COMPOUNDS}$) which explained 61.93% of the total variance (Table 2). Aggression scores were correlated with $PC1_{POST\text{-}AGG\ COMPOUNDS}$ in long-day males, but were not correlated in short-day males (Table 3). Pre-aggression T was correlated with $PC1_{PRE\text{-}AGG\ COMPOUNDS}$ and $PC1_{POST\text{-}AGG\ COMPOUNDS}$ in long-day males, but was not correlated in short-day males (Table 4). These correlations between aggression, T and PCs for urinary compounds provide unbiased evidence that individual compounds that loaded strongly onto those components are altered following an aggression trial and may be regulated by T. There were no other significant relationships between aggression, pre- and post-aggression T and urinary compound PCs (Tables 3 and 4).

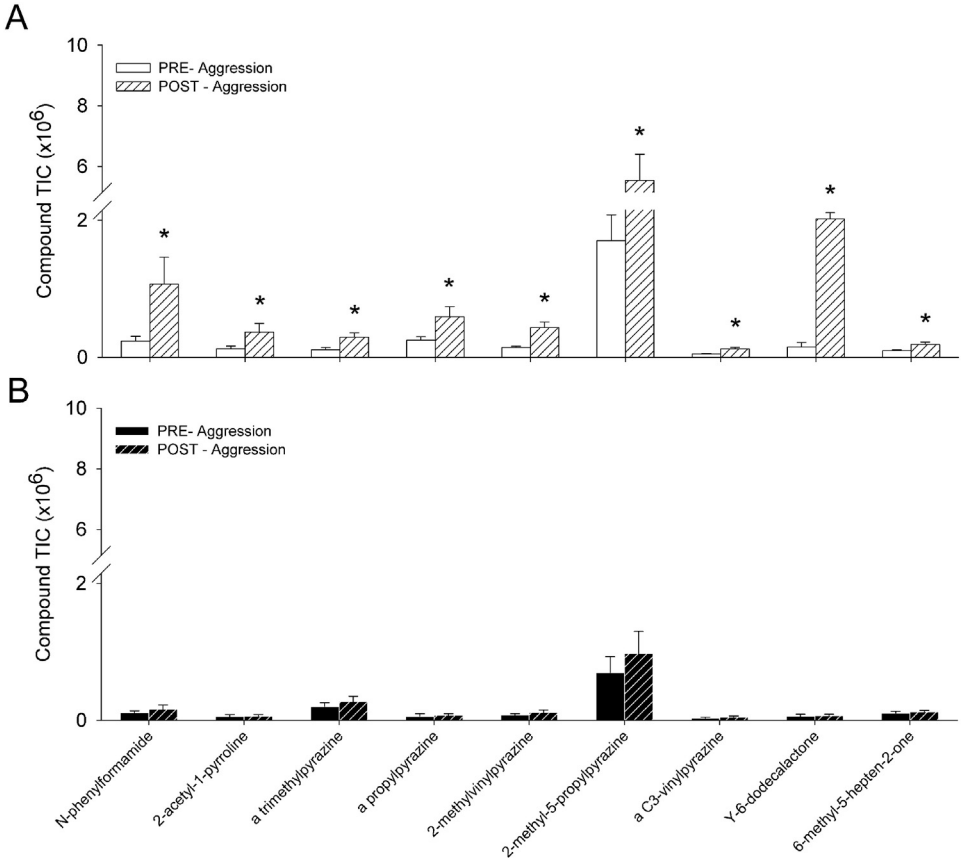

Fig. 2. Urinary compounds were altered after an aggressive encounter in long-day males. Levels of pre-aggression and post-aggression urinary compounds for (A) long days; and (B) short days. *Statistically significantly different (repeated-measures ANOVAs followed by paired *t*-tests, $p < 0.05$).

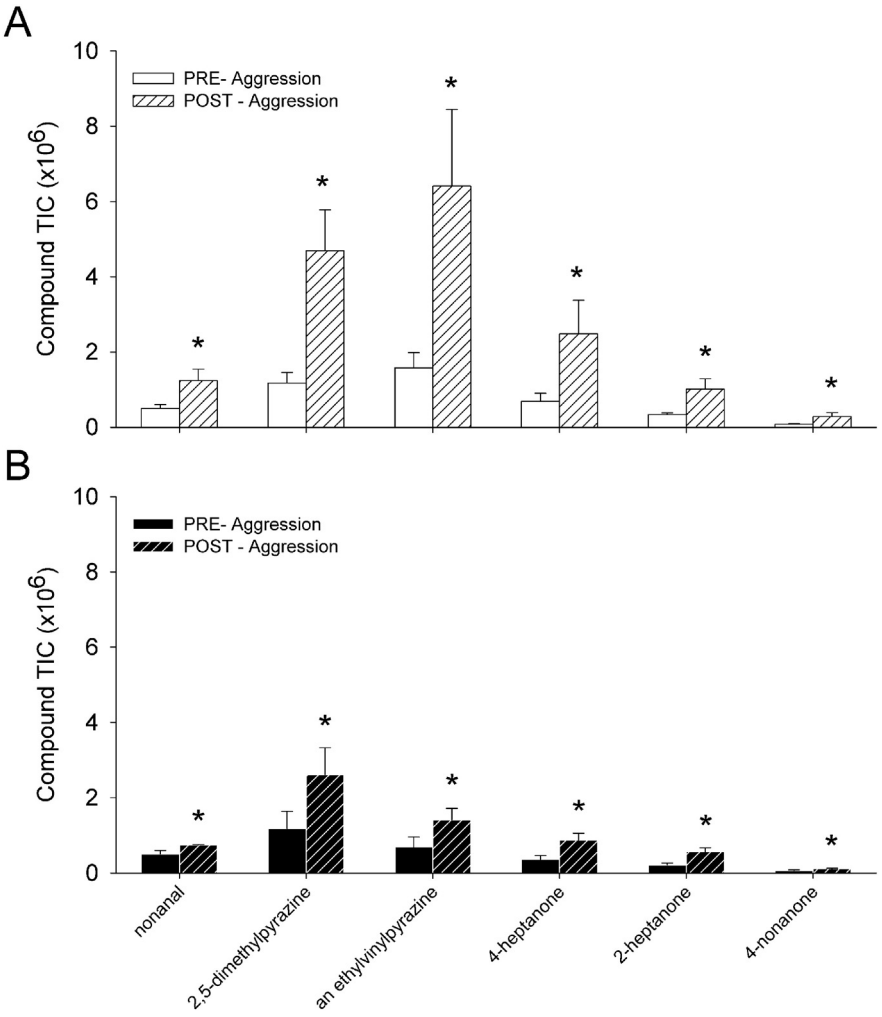

Fig. 3. Urinary compounds were altered after an aggressive encounter in males across photoperiods. Levels of pre-aggression and post-aggression urinary compounds for (A) long days; and (B) short days. *Statistically significantly different (repeated-measures ANOVAs followed by paired *t*-tests, $p < 0.05$).

4. Discussion

In the present study, we identified individual hamster urinary compounds using stir bar sorptive extractions (SBSE), a highly efficient sampling method suitable for trace level analysis of VOCs in a complex biological matrix. Using this method, we measured compounds in individual males, as well as determined the effects of reproductive phenotype and aggression on levels of these compounds. Siberian hamster urine included a broad range of chemical compounds, both in terms of the number of the chemical classes as well as structural diversity within each class. Moreover, both photoperiod and aggression affected urinary compounds. Long-day males generally had elevated levels and also displayed more photoperiodic- and aggression-induced changes in specific compounds, when compared to short-day males. There were also urinary compounds that increased after an aggressive encounter irrespective of photoperiod. Further, aggression and serum T were correlated with urinary compounds in long but not short days. These findings suggest photoperiod- and aggression-specific physiological regulation of urinary VOC excretion in this species.

4.1 Hamster urinary compounds have a distinct chemical signature

We identified 39 urinary compounds representing eight chemical classes (categorized on the basis of overall structure and according to functional groups with the same or similar modes of metabolism). The chemical constituents of urine and the ventral gland are strikingly different [25,37]. Male hamster urinary compounds are notably composed of a group of pyrazines, which are among the most odoriferous substances. The dominating presence of pyrazines has previously been reported in male Siberian hamster urine and is known to be most lacking in females [29]. Further, the three most elevated compounds under the different conditions in this study were 2-methyl-5-propylpyrazine, 2,5-dimethylpyrazine and an ethylvinylpyrazine, which are likely male-specific compounds [29]. Ketones were also found as a predominate chemical signature in urine consistent with previous findings [29].

In contrast to urine, the majority of ventral gland constituents were carboxylic acids, alcohols and ketones. In addition, there were several male-specific unsaturated ketones, including 4,8-dimethyl-3,8-nonadien-2-one, which may be unique to this species [25,37,38]. There were also two female-specific methyl ketones, 2-dodecanone and 2-tetradecanone [25,37,38]. Siberian hamsters have been reported to use urine and ventral gland secretions as scent marks to delineate their respective territorial boundaries, therefore the lack of chemical overlap suggests that these scent profiles convey different information among senders and receivers, which are likely dependent on glandular source of origin [22,24]. Ventral gland compounds have also been shown to be involved in aggressive encounters as they change with reproductive phenotype and are associated with short-day increases in aggression [25]. Our results here nicely parallel these previous findings and suggest that urine is used more during periods of reproductive activity, consistent with previous observations in hamsters in wild and laboratory settings [21,24,29]. In addition to urinary and ventral gland

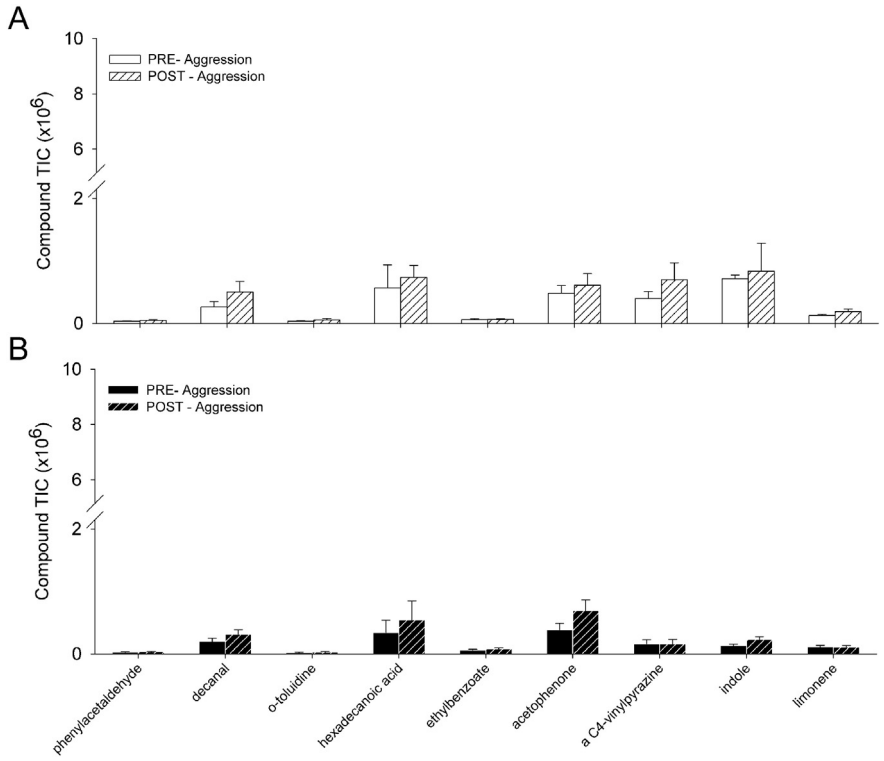

Fig. 4. Urinary compounds were not altered after an aggressive encounter in males. Levels of pre-aggression and post-aggression urinary compounds for (A) long days; and (B) short days. *Statistically significantly different (repeated-measures ANOVAs followed by paired *t*-tests, $p < 0.05$).

excretions, both male and female hamsters excrete from their supplementary sacculi glands, which are at the opening of the cheek pouches. These secretions are chemically distinct from other glandular secretions and do not seem to be involved in territorial defense [38]. Instead, buccal secretions are associated with parent-pup interactions and seem critical for successful development in early life [39].

4.2 Hamster urinary compounds and other rodent and mammalian urinary metabolites

In a previous study that compared pooled urine samples of three species in the *Phodopus* genus Siberian hamsters and Campbell's hamsters (*Phodopus campbelli*) were very similar with regards to pyrazine

Table 2
Principal components loading and eigenvalues for male pre- and post-aggression urinary compounds. Bold values indicate that variables loaded strongly within the component (≤−0.5 or ≥0.5).

Compounds	$PC1_{PRE\text{-}AGG\ COMPOUNDS}$	$PC2_{PRE\text{-}AGG\ COMPOUNDS}$	$PC1_{POST\text{-}AGG\ COMPOUNDS}$	$PC2_{POST\text{-}AGG\ COMPOUNDS}$
phenylacetaldehyde	0.33	−0.11	0.45	−0.25
nonanal	**0.78**	−0.34	**0.73**	**0.54**
decanal	**0.53**	−0.10	**0.56**	−0.44
N-phenylformamide	**0.53**	**0.61**	**0.72**	−0.28
o-toluidine	**0.72**	0.26	**0.58**	−0.45
hexadecanoic acid	0.04	−0.16	0.02	0.31
ethyl benzoate	**0.73**	−0.33	0.42	**0.72**
2,5-dimethylpyrazine	**0.80**	−0.06	**0.77**	−0.29
2-acetyl-1-pyrroline	0.26	**0.57**	**0.65**	−0.29
trimethylpyrazine	**0.76**	−0.26	0.30	0.16
propylpyrazine	0.46	**0.76**	**0.81**	−0.45
2-methylvinylpyrazine	**0.69**	0.44	**0.83**	−0.25
acetophenone	**0.68**	−0.14	**0.66**	**0.70**
2-methyl-5-propylpyrazine	**0.80**	0.46	**0.79**	−0.31
an ethylvinylpyrazine	**0.71**	0.42	**0.67**	−0.36
a C3-vinylpyrazine	−0.05	−0.17	**0.79**	−0.17
a C4-vinylpyrazine	0.19	**0.51**	**0.70**	−0.37
indole	**0.65**	**−0.58**	**0.60**	**0.75**
γ-6-dodecalactone	−0.03	−0.10	0.33	**0.66**
4-heptanone	0.22	−0.07	**0.80**	0.42
2-heptanone	**0.60**	0.29	**0.87**	0.07
6-methyl-5-hepten-2-one	**0.82**	−0.45	**0.61**	−0.30
4-nonanone	**0.78**	−0.35	**0.78**	0.38
limonene	**0.56**	−0.47	**0.71**	0.43
Eigenvalue	8.36	3.55	10.49	4.37
% variance explained	34.65	14.80	43.73	18.20

Table 3
Spearman's rank correlations between aggression scores, $PC1_{PRE\text{-}AGG\ COMPOUNDS}$, $PC2_{PRE\text{-}AGG\ COMPOUNDS}$, $PC1_{POST\text{-}AGG\ COMPOUNDS}$ and $PC2_{POST\text{-}AGG\ COMPOUNDS}$ in male hamsters. Significant *p*-values are shown in bold.

Group	*n*	Aggression and $PC1_{PRE\text{-}AGG\ COMPOUNDS}$		Aggression and $PC2_{PRE\text{-}AGG\ COMPOUNDS}$		Aggression and $PC1_{POST\text{-}AGG\ COMPOUNDS}$		Aggression and $PC2_{POST\text{-}AGG\ COMPOUNDS}$	
		ρ	*p*	ρ	*p*	ρ	*p*	ρ	*p*
Long days	12	0.28	0.09	0.13	0.68	0.29	**0.02**	0.06	0.86
Short days	16	−0.22	0.41	−0.19	0.47	0.20	0.45	0.33	0.21

and ketone profiles [29]. Specifically, both species expressed similar levels of each compound, however, Campbell's hamsters displayed more male-specific pyrazines. Although very closely related, these species differ in the degree of paternal care given; Campbell's hamsters display paternal care whereas Siberian hamsters do not. One possibility is that male-specific pyrazines function to advertise male quality, but the information content may differ due to their respective mating systems. The precise information conveyed by these VOCs, however, is not yet known. Roborovski hamsters (*Phodopus roborovskii*) have a urinary profile composed of substituted quinoxalines, which is completely different than that observed in Siberian hamsters and Campbell's hamsters, suggesting that the metabolic processes underlying urinary compounds likewise drastically differ among these species [29].

When examining other rodents, male and female pine voles (*Microtus pinetorum*) and deer mice (*Peromyscus maniculatus*) share some common compounds, such as pyrazines and ketones, in the urinary profiles with Siberian hamsters and Campbell's hamsters [29,40,41]. However, in contrast to hamsters, female deer mice display elevated levels of pyrazines when compared to male deer mice. These data suggest an important role of pyrazines in rodent chemical communication and demonstrate their multi-faceted roles among species and sexes. Pyrazines have diverse roles and signaling functions among insects, vertebrates, fungi and bacteria [42]. Hamsters, deer mice and many other rodents, however, differ markedly with respect to the urinary profiles of house mice, *Mus domesticus*. For example, Siberian hamsters have a different chemical makeup and considerably fewer chemical constituents than the house mouse, which contains a repertoire of over 100 chemicals [2,43]. Further, female mouse urine contains 2,5-methylpyrazine with the distinct biological activity, in this species, to delay puberty in both female and male juvenile mice [44,45]. In addition to investigating qualitative differences among Siberian hamster urinary profiles, we examined whether compound levels play a role within a seasonal and aggressive context.

4.3 Aggression and urinary compounds

A key finding in this study is that individual urinary compounds were *increased* following a same-sex aggressive encounter and further, overall changes in urinary compounds following this social challenge were associated with individual levels of aggressiveness. Six urinary compounds were altered including two pyrazines and three ketones (*e.g.*, 2,5-dimethylpyrazine and 4-nonanone). Although we cannot completely rule out that changes in urinary compounds were influenced by other factors besides the aggressive interaction (*e.g.*, handling), this is unlikely because of the high reproducibility within individuals and across time in non-social encounters. We also report photoperiod-specific increases in select compounds in response to aggression; nine urinary compounds were increased in long days but not short days. Interestingly, the compounds induced in long days were predominately pyrazines (*e.g.*, 2-methyl-5-propylpyrazine) that have previously been described as male-specific compounds present in adults, but absent or present at low levels in juvenile males [29]. Although males across both photoperiods displayed changes in urinary compounds in response to aggression, long-day males showed a much more robust magnitude of change compared with short-day males. The magnitude of change in the levels of individual urinary compounds before and after aggression, however, did not parallel the intensity of aggression; urinary compounds were altered most notably in long-day hamsters, which display decreased aggression compared with short-day hamsters [18,20]. At a mechanistic level, these distinct changes in urinary compounds in response to aggression suggest that different metabolic cascades may be responsible for changes to the urinary profile across photoperiods; however, such mechanisms explaining this pattern are currently unknown. While we did not explicitly examine urinary compounds at a functional level, these results suggest that urinary compounds may play a greater role within a long-day context when animals are reproductively competent and less in short days when reproduction is inhibited. In contrast, short-day animals appear to shift to the use of ventral gland signaling during aggressive encounters [25]. Taken together, these findings support the idea of an adjustment in signaling across photoperiods. Additional studies will be needed to conduct functional analyses of candidate urinary volatile compounds identified here in order to gain a greater understanding of their role in the chemical communication of this species.

4.4 Reproductive phenotype and urinary compounds

Another finding of note in the present study is that changes in reproductive phenotype, in response to photoperiod regimes, contributed to

Table 4
Spearman's rank correlations between pre- and post-aggression T, $PC1_{PRE\text{-}AGG\ COMPOUNDS}$, $PC2_{PRE\text{-}AGG\ COMPOUNDS}$, $PC1_{POST\text{-}AGG\ COMPOUNDS}$ and $PC2_{POST\text{-}AGG\ COMPOUNDS}$ in male hamsters. Significant *p*-values are shown in bold.

Group	*n*	Pre-aggression T and $PC1_{PRE\text{-}AGG}$ COMPOUNDS		Post-aggression T and $PC1_{PRE\text{-}AGG}$ COMPOUNDS		Pre-aggression T and $PC2_{PRE\text{-}AGG}$ COMPOUNDS		Post-aggression T and $PC2_{PRE\text{-}AGG}$ COMPOUNDS	
		ρ	*p*	ρ	*p*	ρ	*p*	ρ	*p*
Long days	12	−0.59	**0.03**	−0.04	0.91	0.31	0.32	0.24	0.46
Short days	16	0.23	0.39	0.16	0.56	−0.25	0.35	−0.36	0.17

	Pre-aggression T and $PC1_{POST\text{-}AGG}$ COMPOUNDS		Post-aggression T and $PC1_{POST\text{-}AGG}$ COMPOUNDS		Pre-aggression T and $PC2_{POST\text{-}AGG}$ COMPOUNDS		Post-aggression T and $PC2_{POST\text{-}AGG}$ COMPOUNDS	
	ρ	*p*	ρ	*p*	ρ	*p*	ρ	*p*
	−0.57	**0.04**	−0.38	0.22	0.10	0.75	−0.02	0.95
	−0.39	0.14	−0.25	0.34	0.12	0.66	0.31	0.24

substantial changes in levels of individual compounds. In general, elevated levels of compounds were observed in long days. Baseline levels of pyrazines and ketones were secreted in very high concentrations in long-day hamsters, compared with the moderate levels observed in short-day hamsters. The metabolic processes explaining elevated levels of urinary compounds as well as a larger number of compounds that change in response to behavior for long-day animals are likely dependent on high levels of gonadal steroids reported here and previously [18,32]. We show a long-day specific association between levels of baseline T and levels of urinary compounds that loaded onto the first principal component. Most urinary compounds that loaded strongly onto the first component also show elevations during long days (*e.g.*, 2-methyl-5-propylpyrazine and an ethylvinylpyrazine). Such a strong association during long days suggests a role for T in the regulation of these urinary compounds. Because most, but not all, urinary compounds are associated with T, however, this suggests that these other compounds may be regulated by other gonadal steroids or by a T-independent mechanism [46].

Many urinary compounds that loaded strongly onto the first component did not show photoperiodic variation (*e.g.*, 2-acetyl-1-pyrroline and γ-6-dodecalactone), however, showed aggression-induced increases that simultaneously occurred with aggression-induced decreases in T during long days. This marked decrease in T concomitant with marked increases in urinary constituents suggests that T is involved in the onset of metabolic conversion of these compounds. In contrast, changes following aggressive encounters, irrespective of photoperiod, suggest that there are T- or gonadal steroid-independent effects on these chemical compounds. Modest changes in T, site-specific metabolic conversion, or androgen receptor expression and kinetics were not captured by our quantification of baseline T [46]. In addition, we did not examine non-gonadal mechanisms of aggression by which these urinary compounds could also be regulated [46]. To further explore the relationship between urinary compound production, gonadal steroids and T, studies should combine the natural fluctuations of reproductive phenotype with experimental manipulations of reproductive phenotype such as gonadectomies and hormone replacement. In addition, future studies should test the likely non-gonadal mechanisms by which aggression and a subset of urinary compounds are regulated. Such approaches are the critical next steps needed to gain a greater understanding of the underlying mechanisms regulating chemical communication of this species.

5. Conclusions

We show that different photoperiod conditions and aggressive challenges influence variation in levels of specific compounds across the broad range of urinary profiles in Siberian hamsters. These chemical signals, used for territory marking, also share some of the urinary compound composition with other rodents, although the function of the same chemical compound may differ between species. Further, differences across photoperiods in relative levels of urinary compounds as well as photoperiod-dependent and photoperiod-independent changes in urinary compounds following behavioral trials make Siberian hamsters an excellent species in which to explore both the functional significance and physiological regulation of these compounds in future studies. Our data illustrate the important role of urinary compounds in regulating hamster chemical communication [25,29,37,47] and communication in general [48,49]. Collectively, these findings advance our understanding of mammalian chemical communication and social behavior more comprehensively.

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Appendix A. Supplementary material

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References

[1] J.W. Bradbury, S.L. Vehrencamp, Principles of Animal Communication, 2 ed. Sinauer Associates, Inc., 2011
[2] S.D. Liberles, Mammalian pheromones, Annu. Rev. Physiol. 76 (2014) 151–175.
[3] R. Tirindelli, M. Dibattista, S. Pifferi, A. Menini, From pheromones to behavior, Physiol. Rev. 89 (2009) 921–956.
[4] R.E. Johnston, Chemical communication in rodents: from pheromones to individual recognition, J. Mammal. 84 (2003) 1141–1162.
[5] P. Chamero, V. Katsoulidou, P. Hendrix, B. Bufe, R. Roberts, H. Matsunami, et al., G protein Gαo is essential for vomeronasal function and aggressive behavior in mice, Proc. Natl. Acad. Sci. 108 (2011) 12898–12903.
[6] K. Del Punta, T. Leinders-Zufall, I. Rodriguez, D. Jukam, C.J. Wysocki, S. Ogawa, et al., Deficient pheromone responses in mice lacking a cluster of vomeronasal receptor genes, Nature 419 (2002) 70–74.
[7] L. Stowers, T.E. Holy, M. Meister, C. Dulac, G. Koentges, Loss of sex discrimination and male-male aggression in mice deficient for TRP2, Science 295 (2002) 1493–1500.
[8] M. Novotny, S. Harvey, B. Jemiolo, J. Alberts, Synthetic pheromones that promote inter-male aggression in mice, Proc. Natl. Acad. Sci. 82 (1985) 2059–2061.
[9] M. Novotny, S. Harvey, B. Jemiolo, Chemistry of male dominance in the house mouse, *Mus domesticus*, Experientia, 46 1990, pp. 109–113.
[10] B. Jemiolo, T.-M. Xie, M. Novotny, Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals, Physiol. Behav. 50 (1991) 1119–1122.
[11] C.M. Drea, D'scent of man: a comparative survey of primate chemosignaling in relation to sex, Horm. Behav. 68 (2015) 117–133.
[12] R. Apfelbach, H.A. Soini, N.Y. Vasilieva, M.V. Novotny, Behavioral responses of predator-naïve dwarf hamsters (*Phodopus campbelli*) to odor cues of the European ferret fed with different prey species, Physiol. Behav. 146 (2015) 57–66.
[13] L.K. Greene, K.E. Grogan, K.N. Smyth, C.A. Adams, S.A. Klager, C.M. Drea, Mix it and fix it: functions of composite olfactory signals in ring-tailed lemurs, Royal Society Open Science 3 (2016) 160076.
[14] J.C. Crawford, C.M. Drea, Baby on board: olfactory cues indicate pregnancy and fetal sex in a non-human primate, Biol. Lett. 11 (2015) 20140831.
[15] A. Petrulis, Chemosignals and hormones in the neural control of mammalian sexual behavior, Front. Neuroendocrinol. 34 (2013) 255–267.
[16] H. Arakawa, D.C. Blanchard, K. Arakawa, C. Dunlap, R.J. Blanchard, Scent marking behavior as an odorant communication in mice, Neurosci. Biobehav. Rev. 32 (2008) 1236–1248.
[17] J.L. Hurst, C.E. Payne, C.M. Nevison, A.D. Marie, R.E. Humphries, D.H. Robertson, et al., Individual recognition in mice mediated by major urinary proteins, Nature 414 (2001) 631–634.
[18] A.M. Jasnow, K.L. Huhman, T.J. Bartness, G.E. Demas, Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*), Horm. Behav. 38 (2000) 102–110.
[19] F. Scherbarth, S. Steinlechner, Endocrine mechanisms of seasonal adaptation in small mammals: from early results to present understanding, J. Comp. Physiol. B. 180 (2010) 935–952.
[20] N.M. Rendon, L.M. Rudolph, D.R. Sengelaub, G.E. Demas, The agonistic adrenal: melatonin elicits female aggression via regulation of adrenal androgens, Proc. R. Soc. B Biol. Sci. 282 (2015) 20152080.
[21] N.Y. Feoktistova, Sex discrimination by different odors in the dwarf hamsters: *Phodopus roborovskii* and *Phodopus sungorus*, Adv. Biosci. 93 (1994) 333–338.
[22] K.E. Wynne-Edwards, From dwarf hamster to daddy: the intersection of ecology, evolution, and physiology that produces paternal behavior, Adv. Study Behav. 32 (2003) 207–262.
[23] K.E. Wynne-Edwards, R.D. Lisk, Behavioral interactions differentiate djungarian (*Phodopus campbelli*) and Siberian (*Phodopus sungorus*) hamsters, Can. J. Zool. 65 (1987) 2229–2235.
[24] K.E. Wynne-Edwards, A.V. Surov, A.Y. Telitzina, Field studies of chemical signalling: direct observations of dwarf hamsters (*Phodopus*) in Soviet Asia, Chemical Signals in Vertebrates 6, Springer 1992, pp. 485–491.
[25] N.M. Rendon, H.A. Soini, M.-A.L. Scotti, E.R. Weigel, M.V. Novotny, G.E. Demas, Photoperiod and aggression induce changes in ventral gland compounds exclusively in male Siberian hamsters, Horm. Behav. 81 (2016) 1–11.
[26] V. Sokolov, N. Feoktistova, Response of adult djungarian hamsters (*Phodopus sungorus*) to odor signals of species of their own and opposite sex during different seasons with the reference to reproduction process, Izv. Akad. Nauk Ser. Biol. (1997) 369–373 (Rossiiskaia akademiia nauk).
[27] S.J. Scribner, K.E. Wynne-Edwards, Moderate water restriction differentially constrains reproduction in two species of dwarf hamster (*Phodopus*), Can. J. Zool. 72 (1994) 1589–1596.

[28] M. Trojan, Comparative investigations on the water budget and the kidney function in the Palaearctic hamsters *Cricetus cricetus* (Leske, 1779), *Mesocricetus auratus* (Waterhouse, 1893), *Cricetulus griseus* (Milne-Edwards, 1867) and *Phodopus sungorus* (Pallas, 1770), Zoologische Jahrbucher-Abteilung Fur Allgemeine Zoologie Und Physiologie Der Tiere, 83 1979, pp. 192–223.
[29] H.A. Soini, D. Wiesler, R. Apfelbach, P. König, N.Y. Vasilieva, M.V. Novotny, Comparative investigation of the volatile urinary profiles in different *Phodopus* hamster species, J. Chem. Ecol. 31 (2005) 1125–1143.
[30] M.R. Gorman, I. Zucker, Testicular regression and recrudescence without subsequent photorefractoriness in Siberian hamsters, Am. J. Physiol. Regul. Integr. Comp. Physiol. 38 (1995) (R800-R6).
[31] G.R. Lynch, C.B. Lynch, R.M. Kliman, Genetic analyses of photoresponsiveness in the djungarian hamster, *Phodopus sungorus*, J. Comp. Physiol. A. 164 (1989) 475–481.
[32] T.A. Bedrosian, L.K. Fonken, G.E. Demas, R.J. Nelson, Photoperiod-dependent effects of neuronal nitric oxide synthase inhibition on aggression in Siberian hamsters, Horm. Behav. 61 (2012) 176–180.
[33] L. Stowers, T.-H. Kuo, Mammalian pheromones: emerging properties and mechanisms of detection, Curr. Opin. Neurobiol. 34 (2015) 103–109.
[34] J.L. Hurst, R.J. Beynon, Scent wars: the chemobiology of competitive signalling in mice, Bioessays 26 (2004) 1288–1298.
[35] M. Novotny, Pheromones, binding proteins and receptor responses in rodents, Biochem. Soc. Trans. 31 (2003) 117–122.
[36] C. Lessells, P.T. Boag, Unrepeatable repeatabilities: a common mistake, Auk 104 (1987) 116–121.
[37] B. Burger, D. Smit, H. Spies, C. Schmidt, U. Schmidt, A. Telitsina, et al., Mammalian exocrine secretions XV. Constituents of secretion of ventral gland of male dwarf hamster, *Phodopus sungorus sungorus*, J. Chem. Ecol. 27 (2001) 1259–1276.
[38] B.V. Burger, Mammalian semiochemicals, The Chemistry of Pheromones and Other Semiochemicals II, Springer 2005, pp. 231–278.
[39] R. Apfelbach, U. Schmidt, N.Y. Vasilieva, The secretion of the supplementary sacculi of the dwarf hamster *Phodopus campbelli*, Chemical Signals in Vertebrates 9, Springer 2001, pp. 347–352.
[40] W. Ma, D. Wiesler, M.V. Novotny, Urinary volatile profiles of the deermouse (*Peromyscus maniculatus*) pertaining to gender and age, J. Chem. Ecol. 25 (1999) 417–431.
[41] M.L. Boyer, B. Jemiolo, F. Andreolini, D. Wiesler, M. Novotny, Urinary volatile profiles of pine vole, *Microtus pinetorum*, and their endocrine dependency, J. Chem. Ecol. 15 (1989) 649–662.
[42] A. Woolfson, M. Rothschild, Speculating about pyrazines, Proc. R. Soc. Lond. B Biol. Sci. 242 (1990) 113–119.
[43] F. Schwende, D. Wiesler, J. Jorgenson, M. Carmack, M. Novotny, Urinary volatile constituents of the house mouse, *Mus musculus*, and their endocrine dependency, J. Chem. Ecol. 12 (1986) 277–296.
[44] B. Jemiolo, M. Novotny, Inhibition of sexual maturation in juvenile female and male mice by a chemosignal of female origin, Physiol. Behav. 55 (1994) 519–522.
[45] M. Novotny, B. Jemiolo, S. Harvey, D. Wiesler, A. Marchlewska-Koj, Adrenal-mediated endogenous metabolites inhibit puberty in female mice, Science 231 (1986) 722–725.
[46] K.K. Soma, N.M. Rendon, R. Boonstra, H.E. Albers, G.E. Demas, DHEA effects on brain and behavior: insights from comparative studies of aggression, J. Steroid Biochem. Mol. Biol. 145 (2015) 261–272.
[47] B. Burger, D. Smit, H. Spies, C. Schmidt, U. Schmidt, A. Telitsina, Mammalian exocrine secretions XVI. Constituents of secretion of supplementary sacculi of dwarf hamster, *Phodopus sungorus sungorus*, J. Chem. Ecol. 27 (2001) 1277–1288.
[48] S.M. Keesom, N.M. Rendon, G.E. Demas, L.M. Hurley, Vocal behaviour during aggressive encounters between Siberian hamsters, *Phodopus sungorus*, Anim. Behav. 85-93 (2015).
[49] N.M. Rendon, S.M. Keesom, C. Amadi, L.M. Hurley, G.E. Demas, Vocalizations convey sex, seasonal phenotype, and aggression in a seasonal mammal, Physiol. Behav. 152 (2015) 143–150.