

## Females of the communally breeding rodent, *Octodon degus*, transfer antibodies to their offspring during pregnancy and lactation

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### Abstract

Females in numerous rodent species engage in communal nesting and breeding, meaning that they share a nest to rear their young together. One potential benefit to communally nesting mothers is that infants improve their immunocompetence. Thus, suckling from two or more females might provide newborns with a more diverse array of antibodies and defensive cells. As a first step toward testing the immunocompetence hypothesis, we assessed whether female degus (*Octodon degus*), a communally nesting and breeding caviomorph rodent, transfer immunoglobulins to their young through the yolk sac or placenta while in the uterus and, during lactation, through milk. With this aim, adult degu females were immunized with four antigens, including two mollusk hemocyanins from *Concholepas* and *Megathura* (CCH and KLH, respectively), porcine thyroglobulin and tetanus toxoid. Specific antibodies against the experimental antigens were used to track the origin of antibodies in the young. To establish the presence of specific antibodies of IgG and IgA isotypes in sera and milk of animals, an indirect enzyme-linked immunosorbent assay (ELISA) was developed. Degu females produced specific antibodies against antigens not found in their natural environment, and mothers were able to transfer the induced antibodies to their litters during pregnancy (IgG) and during lactation (IgA). However, we recorded only limited evidence of degu offspring acquiring antibodies from lactating mothers other than their own, giving little support to the increased immunocompetence hypothesis.

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**Keywords:** Communal nesting and breeding; Immunity transfer; Immunoglobulins; Pregnancy; Lactation; Degus

### 1. Introduction

Individuals of numerous rodent species engage in group-living, meaning that they share an area of activity,

a nest (or den), and interact more frequently with group members than with individuals from other groups. When group members share a nest during breeding and rear their young together, they are regarded as communal breeders (Hayes, 2000; Lewis and Pusey, 1997; Solomon and Getz, 1997). Under these conditions, female breeders may incur a cost of providing milk to less related or totally unrelated offspring (Hayes, 2000). The provision

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of milk to unrelated offspring is costly, given that lactation represents the most energetically expensive cost of breeding rodents and mammals (Thompson, 1992), and lactating females pay a fitness cost in terms of subsequent survival and reproductive success (Clutton-Brock et al., 1989). Providing milk and other parental resources to unrelated offspring may involve additional short-term costs such as increased transmission of internal and external pathogens to mothers and pups (Roulin and Heeb, 1999). Therefore, explanations for the occurrence of communal breeding tend to rely on direct and indirect benefits that compensate such drawbacks, including protection of young from infanticide or predation, improved thermoregulation of young, improved growth of young through enhanced milk intake, reduction of maternal energy costs and adoption of young whose mothers die (Hayes, 2000; Lee, 1989; Lewis and Pusey, 1997; Riedman, 1982; Roulin, 2002). Potential benefits, however, include the possibility that infants improve their immunocompetence (Roulin and Heeb, 1999). Thus, suckling from two or more, genetically different, lactating females might provide newborns and young with a more diverse and polyvalent population of immunoglobulins and defensive cells (macrophages, dendritic cells and lymphocytes).

Evidence that young improve immunity through the milk of foster mothers is well known in humans, laboratory primates and mice. Thus, human milk is known to have several cells and molecules that enhance protection from microbes and parasites (Gillin and Reiner, 1983; Newman, 1995). More importantly, protective cells (e.g., leukocytes) and immunoglobulins can resist digestion and cross the intestinal epithelium of neonates (Xanthou, 1998; Van de Perre, 2003). As might be expected, breast-fed infants experience less illness and mortality than formula-fed infants (Dewey, 1998; Villalpando and Hamosh, 1998). In mice, B-cell deficient neonates nursed from B-cell normal foster mothers develop higher levels of serum IgG and splenic B cells (Arvola et al., 2001). More importantly, neonatal mice nursed from immunoglobulin-deficient mothers grow relatively less and suffer high mortality early in life (Gustafsson et al., 1994). The only available study on free-living wild rodents demonstrated that maternal antibodies postpone hantavirus infection and enhance breeding success in the bank vole *Clethrionomys glareolus* (Kallio et al., 2006).

In contrast to that in humans and a few other animal models, very little is known of free-living wild species (Kallio et al., 2006) and communally breeding species in particular. In this study, experiments were designed to investigate whether female degus (*Octodon degus*), a caviomorph rodent from central Chile, are able to achieve

passive transfer of humoral immunity to their litters, therefore, protecting them from pathogens. In particular, we examined whether specific antibodies are acquired through the yolk sac or placenta by young while in uterus, during lactation through the milk, or both. Evidence from mice and rats indicates that some immunoglobulins can be transferred to young through both ways (Carlier and Truysens, 1995). Differing from humans, where most IgG are transmitted to the fetus from amniotic fluid through the vascular system present in the placenta, mice and rats acquire most IgG from colostrum and milk (Rojas and Apodaca, 2002). Furthermore, immunoglobulin transport in rodents, bovines, cats and ferrets takes place across the intestinal epithelium into the neonatal circulation (Van de Perre, 2003). This transfer is carried out by enterocytes located in intestinal crypts and on the surface of villi that express surface membrane FcRn receptors that bind IgG to facilitate their transcytosis (Rojas and Apodaca, 2002).

Degus are social rodents where a variable number of females (one to four) and one or two males share one or more underground burrow systems (Ebensperger et al., 2004; Fulk, 1976). More importantly, communally nesting groups include simultaneously lactating females (Ebensperger et al., 2004), and allonursing of young has been observed among captive individuals (Ebensperger et al., 2002). More recently, establishment of communal litters and allonursing were all recorded in captivity under conditions resembling natural settings, including a limited supply of food, but under an unlimited supply of nest boxes, i.e., burrows (Ebensperger et al., 2007). Intriguingly, this study revealed that some females within communal nests pay an immediate direct fitness cost in terms of reduced growth and survival of their pups to weaning age (Ebensperger et al., 2007). Therefore, the possibility that communally breeding females accrue other long-term fitness benefits, such as the enhancement of immunocompetence, to their offspring needs to be examined.

With this aim, we immunized adult degu females with four protein antigens, including two mollusk hemocyanins (one from *Concholepas concholepas* or CCH, and one from *Megathura crenulata* or KLH), tetanus toxoid (TT) and porcine thyroglobulin (TYG). We selected these antigens based on hemocyanins being powerful immunogens due to the absence of similar molecules in mammals, their large size and repeated subunit structure (Harris and Markl, 1999; Van Holde and Miller, 1995). Current applications of hemocyanins include their use as carriers for vaccines against pathogens and cancer (Markl et al., 2001; Moltedo et al., 2006), and in contraceptive vaccines to control wild animal populations

(Miller, 2002). While CCH and KLH resemble each other in having two subunits, they differ in quaternary structure (Leyton et al., 2005; De Ioannes et al., 2004; Oliva et al., 2002), and hence, it was of interest to evaluate whether the humoral immune response against them is similar in wild animals such as degus. TT is a human vaccine antigen and its administration to pregnant women causes antibodies to pass to the fetus throughout the placenta providing protection against neonatal tetanus (Sheffield and Ramin, 2004). Finally, TYG has been used widely as a carrier protein and experimental antigen in different mammals (Glass et al., 1990). In this study, specific antibodies against these antigens were determined using cross-reactivity of anti-mouse IgG serum or anti-mouse IgA  $\alpha$ -chain-specific serum with degu Igs. Thus, antibodies were used as tracers to record the transfer of antibodies during pregnancy and lactation.

## 2. Materials and methods

### 2.1. Chemicals, biochemicals and immunochemical reagents

Bovine serum albumin (BSA) and porcine thyroglobulin were obtained from Sigma Aldrich (Saint Louis, Missouri, USA). Hemocyanin from *Concholepas concholepas* (CCH) was provided by Biosonda Corporation (Santiago, Chile). Tetanus toxoid (TT) was provided by the Instituto de Salud Pública (Santiago, Chile). We used goat anti-mouse IgG (H+L) serum, rabbit anti-goat IgG serum conjugated with alkaline phosphatase, and goat anti-mouse IgA  $\alpha$ -chain-specific serum, all purified through immunoaffinity chromatography. Complete and incomplete Freund's adjuvant, keyhole limpet hemocyanins (KLH) from *Megathura crenulata*, BupH-phosphate buffered saline packs (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2), para-nitrophenyl phosphate, and 96-well polystyrene plates were from Pierce (Rockford, Illinois, USA). All chemicals were analytical-grade reagents and the solutions were prepared with Milli-Q water.

### 2.2. Experimental animals

Study subjects were 1-year-old female descendents from adult females caught during July–August 2002 while pregnant at Lampa (33°17'S; 70°53'W), near Santiago, central Chile. Our degu colony was maintained so animals never bred with close kin. Upon weaning (i.e., about 30 days of age), degu subjects were kept in same sex unrelated pairs inside 45 cm  $\times$  23 cm  $\times$  21 cm clear polycarbonate rat cages

with a bedding of hardwood chips, and water and food (rabbit commercial pellet) provided *ad lib*. Animals were kept in a ventilated room exposed to natural photoperiod and ambient temperature until observations ended (yearly minimum =  $13.4 \pm 0.2$  °C; yearly maximum =  $24.9 \pm 0.2$  °C).

### 2.3. Immunization schedule

A total of 23 six-month-old females were immunized with antigens as follows. On day 1, they received intraperitoneally 1 mg of hemocyanins (CCH or KLH) in 250  $\mu$ L sterile phosphate-buffered saline (PBS); on day 23, we repeated a similar immunization. A booster of 1.5 mg CCH or KLH in 250  $\mu$ L PBS intraperitoneal was given on day 112. While a similar schedule and doses were employed with the tetanus toxoid and porcine thyroglobulin antigens, these were emulsified with 250  $\mu$ L complete Freund's adjuvant (CFC) during the primary inoculation, and with incomplete Freund's adjuvant (IFA) during the secondary inoculation; booster immunizations with TT and TYG were given with PBS. The selection of these four antigens was exploratory because no previous data existed on how degus would respond to them. Hemocyanins were known to induce humoral responses in mice without the use of adjuvant (Molledo et al., 2006). In contrast, TT and TYR have been used always with adjuvant. A total of four females were inoculated with CFC, and then with IFC alone, to serve as negative controls (Glass et al., 1990). The assignment of experimental subjects to a given antigen (or control) condition was random, except that we avoided giving the same treatment to any two females housed in the same cage (i.e., cage mates).

Female degus were bled from the retro-orbital plexus prior to immunization to obtain pre-immune control serum. Ten days after the secondary immunization, animals were assessed to determine the presence of specific IgG or IgA antibodies in sera or milk. The cross-reactivity of anti-mouse IgG (H+L) serum or anti-mouse IgA,  $\alpha$ -chain specific serum, to degu Igs was determined by an indirect enzyme-linked immunosorbent assay (ELISA) as described below.

Two mothers and their fetuses, immunized with CCH and TT, were sacrificed and bled around 8–9 weeks of pregnancy to determine transplacental maternal antibody transmission; sera samples were collected and stored at  $-20$  °C until tested. Lactating females and their offspring were bled about days 3 and 30 after parturition; sera collected was stored at  $-20$  °C until use.

To examine milk from breeding females, we first separated litters from their mother during  $\sim$ 30 min. Then,

we softly pressed the mammary glands of females and collected milk with a capillary tube. Our sampling schedule was planned to collect milk from early (ca. day 3 after delivery of litters) and late (after day 15) lactation of breeding females. Lactation takes 20–30 days in degus. We centrifuged samples at 2000 rpm during 5 min at room temperature, and stored samples at  $-20^{\circ}\text{C}$  until immunological essays.

#### 2.4. Assessment of immune response in degus

We used the general ELISA procedure described by Crowther and Abu-Elzein (1980) and modified by Manosalva et al. (2004). As described before, antibodies against experimental antigens were determined using cross-reactivity of anti-mouse IgG or anti-IgA serum with degu Igs. Micro-well polystyrene plates were incubated overnight at  $4^{\circ}\text{C}$  with 100  $\mu\text{L}$  well of 10  $\mu\text{g}$  solution of antigens (CCH, KLH, TT and TYG) in PBS. Plates were blocked with 200  $\mu\text{L}$  well of 1% PBS-bovine serum albumin (BSA) or with 1% PBS casein, during 2–3 h at room temperature. Then, serial two-fold dilutions of the immune sera or milk of degu females (or sera from offspring) in blocking buffer were incubated for 3 h at  $37^{\circ}\text{C}$ . Plates were washed three times with 200  $\mu\text{L}$  0.02% PBS-Tween-20, and then 100  $\mu\text{L}$  well goat anti-mouse IgG serum alkaline phosphatase (ALP)—conjugated diluted 1:2000 in blocking buffer was added to the wells. After incubating for 30 min at room temperature, plates were washed and developed during 30 min at  $37^{\circ}\text{C}$  by adding 100  $\mu\text{L}$  well of 1 mg  $\mu\text{L}$  para-nitrophenyl phosphate (PNPP) in ALP-buffer ( $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ , 0.2 M, pH 9.6). The reaction was stopped with 3N NaOH and read spectrophotometrically at 405 nm.

To examine anti-IgA specific antibodies, we added 100  $\mu\text{L}$ /well of 50  $\mu\text{L}$  goat anti-mouse IgA,  $\alpha$ -chain specific, diluted 1/100 and incubated in the wells at  $37^{\circ}\text{C}$  during 1 h; plates were then washed as described before and 100  $\mu\text{L}$  well of rabbit anti-goat serum ALP conju-

gate diluted 1:2000 in blocking buffer was added to the wells. After incubating for 30 min at room temperature, plates were washed and developed as described above.

To determine the specificity of immunoreactions of anti-IgG and anti-IgA conjugates, our controls included a (1) pre-immune sera from experimental animals, and (2) sera from animals treated with adjuvant only.

#### 2.5. Data analysis

A titer was defined as the reciprocal of the serum dilution showing the half of the maximum absorbance at 405 nm. Statistical comparisons were performed using non-parametric statistics with the use of Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA). Data are presented as means  $\pm$  S.E.

### 3. Results

#### 3.1. The humoral immune response in *O. degus*

With the aim to examine whether female degus passively provide their litters with antibodies, we developed an immunization protocol to establish the occurrence of uterine and milk transfer of immunity to the offspring. Since there are no anti-degu immunoglobulin isotype antisera commercially available, we established first that *O. degus* and *Mus musculus* immunoglobulins exhibited cross-reactivity. We immunized 23 female subjects against antigens not found in their natural environment (Table 1). Of these, 12 females were inoculated intraperitoneally with hemocyanins CCH or KLH, without adjuvants, while 11 other females were inoculated intraperitoneally with either TT or TYG with adjuvants (Table 1). Animals were given a second dose about 20 days later. Upon secondary inoculation with different antigens, we used an indirect ELISA method to establish the presence of specific IgG antibodies in the sera of females. We found titers of about 1:400 dilution against

Table 1  
Body mass, percent females breeding, females breeding communally, litter size and antigen treatment received by *O. degus* subjects

Antigen	Adjuvant	Number of females	Body mass ( $\bar{x} \pm \text{S.E. g}$ ) <sup>a</sup>	Females breeding (%)	Females breeding communally	Litter size ( $\bar{x} \pm \text{S.E.}$ )
CCH	No	6	226.3 $\pm$ 26.6	5 (83.3) <sup>b</sup>	1	4.6 $\pm$ 2.5
KLH	No	6	223.7 $\pm$ 21.9	4 (66.6)	3	8.3 $\pm$ 1.0
TT	Yes	5	224.0 $\pm$ 10.7	4 (80.0) <sup>b</sup>	1	6.0 $\pm$ 2.2
TYG	Yes	6	217.6 $\pm$ 17.1	1 (16.0)	1	5
No	Yes	4	242.1 $\pm$ 15.2	1 (25.0)	0	8

<sup>a</sup> Before mating.

<sup>b</sup> One mother and its fetuses were from this experimental condition were sacrificed and immediately bled at about 8–9 weeks of pregnancy to assess transplacental antibody transmission.

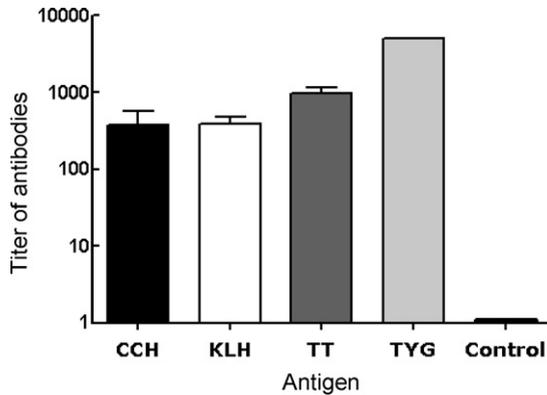


Fig. 1. Secondary humoral immune response in *O. degus* females immunized with: loco (*C. concholepas*) hemocyanin (CCH, without adjuvant;  $n=6$ ), keyhole limpet (*M. crenulata*) hemocyanin (KLH, without adjuvant;  $n=6$ ), tetanus toxoid (TT, with adjuvant;  $n=5$ ) or tyroglobulin (TYG, with adjuvant;  $n=6$ ) and negative controls (adjuvant only;  $n=4$ ). Degu subjects were primed with antigen and, after 23 days, they received a second dose of the same antigen. Samples of sera were taken 10 days after secondary injection and the presence of specific antibodies of the IgG isotype determined by an indirect ELISA. Each bar represents mean antibody titers  $\pm$  S.E., except TYG females that exhibited similarly maximum detectable titers.

CCH and KLH, of 1:1000 dilutions against TT, and of over 1:5000 dilution against TYG (Fig. 1). Titers of different types of antigen did differ (Kruskal–Wallis one-way ANOVA by ranks,  $H_{3,23} = 16.05$ ,  $P=0.011$ ), where females immunized with TYG expressed higher titers (multiple comparisons of mean ranks test,  $P<0.009$ ). Pre-immune sera of experimental degu females did not show reactivity against the corresponding experimental antigen. Control degu (four subjects) inoculated with adjuvants only were all seronegative. One female inoculated with TT and adjuvant, and another exposed to TYG and adjuvant, died during the experiments.

### 3.2. Transuterine passive transfer of immunoglobulins to fetuses

To determine whether degu mothers transfer IgG or IgA antibodies to their fetuses (either through the yolk sac or the placenta), we used indirect ELISA essays to reveal the presence of specific antibodies in the sera of fetuses from 8 to 9 weeks of gestation obtained from two females, one of them immunized with CCH (litter of six pups) and one immunized with TT (litter of seven pups). The observation that fetuses presented IgG antibodies against specific antigens injected into their mothers (Fig. 2) confirmed that transfer of antibodies to fetuses took place. Titters of specific IgG antibodies in the offspring were 1:100 dilution for CCH, and 1:300

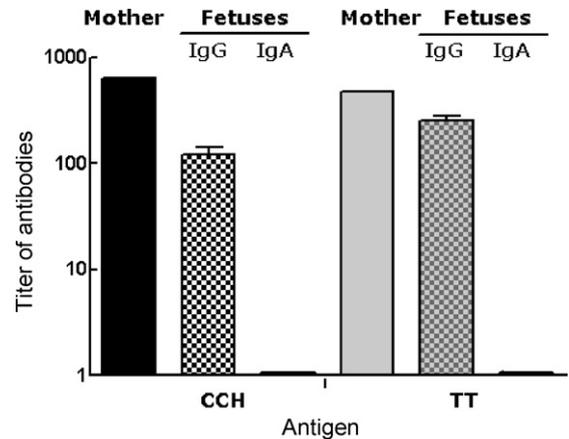


Fig. 2. Transplacental transmission to *O. degus* fetuses from mothers immunized with *C. concholepas* hemocyanin (CCH, one female) or tetanus toxoid (TT, one female). Sera of fetuses of 8–9 weeks of development were obtained and the presence of IgG and IgA specific isotypes against CCH or TT assessed by means of indirect ELISA essays. Each bar represents the antibody titer in the mother and its fetuses  $\pm$  S.E. (six fetuses for CCH mother, and seven fetuses for TT mother).

dilution for TT; the titer in the mothers was about 1:800 for both antigens. More importantly, no IgA were ever detected at this stage (see below).

Data in Fig. 2 demonstrates the specificity of reagents used to determinate degus' Igs. While we were not able to detect IgA in the sera of fetuses, we recorded high concentrations of IgG (the titer was over 100-fold dilutions), indicating that anti-IgA did not cross-react with IgG in degus.

### 3.3. Rate of antibody decay

Since antibodies are catabolized *in vivo*, we investigated decay rates of the specific IgG against antigens in sera of the mothers and neonates. The level of specific serum antibodies in mothers immunized with CCH ( $n=3$ ) and KLH ( $n=2$ ) decreased by 53 and 75%, respectively, from days 214 to 245 ( $\pm 4$ ) after the last immunization (Fig. 3A and B). For subjects immunized with adjuvant, specific antibodies of females injected with TT ( $n=1$ ) decreased by 63% between days 214 and 245 (Fig. 3C). In the case of the single female immunized with TYG that bred ( $n=1$ ), antibodies did not vary between days 223 and 230 of lactation (Fig. 3D). Thus, titers of females immunized without adjuvant (CCH and KLH;  $62 \pm 15\%$ ) tended to decrease more than titers of TT and TYG females ( $32 \pm 31\%$ ), but not significantly so (Mann–Whitney  $U$ -test,  $Z_{adj} = 1.42$ ;  $P=0.155$ ). The level of specific antibodies decayed in

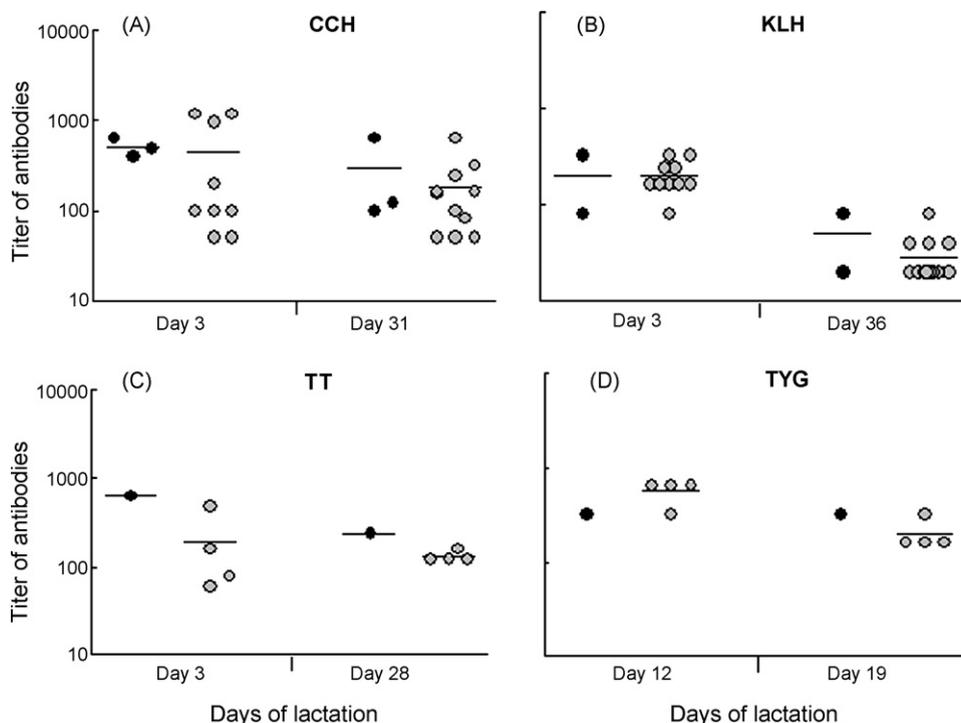


Fig. 3. Decay of maternally transferred antibodies (IgG) in offspring of *O. degus* females immunized with: (A) *C. concholepas* hemocyanin (CCH), (B) keyhole limpet hemocyanin (KLH), both without adjuvant, (C) tetanus toxoid (TT), (D) tyroglobulin (TYG), with Freund's complete and incomplete adjuvant, respectively. The sera of each litter during two moments after birth was taken (abscise), and the presence of specific antibodies against the antigen used to immunize the mother were determined by the indirect ELISA essays. Data were taken during early (ca. day 3) and late (after day 15) lactation of breeding females. Each black dot represents the antibody titer in the sera of mothers while grey dots identify antibody titers of pups ( $\pm$ S.E.).

litters as well (Fig. 3). Antibodies in the offspring of females immunized with CCH ( $n=2$  litters) and KLH ( $n=2$  litters) decreased by 72 and 83%, respectively. In the case of litters from females immunized with TT ( $n=1$  litter) and TYG ( $n=1$  litter), antibody titers decreased by 33 and 71% of initial titers, respectively. Titers in pups whose mothers were immunized without adjuvant tended to decrease more ( $66 \pm 16\%$ ) than titers of pups whose mothers were immunized with adjuvant ( $52 \pm 19\%$ ), a non-statistically significant difference (Mann–Whitney  $U$ -test,  $Z_{\text{adj}}=0.93$ ;  $P=0.355$ ).

### 3.4. IgG and IgA transfer to offspring during lactation

To determine whether transfer of IgG and IgA isotypes to degu litters takes place during lactation as well, we tested the presence of antibodies in milk of immunized females and in serum of litters ca. day 3 of lactation by indirect ELISA essays. We found that, for all antigens, both immunoglobulin isotypes were present in the milk (Fig. 4A). Titers of IgA for all antigens averaged

$210 \pm 48$ , while those of IgG averaged  $26 \pm 6$ , a statistically significant difference (Wilcoxon matched pairs test,  $Z=2.02$ ;  $P=0.0431$ ). While IgA were more abundant than IgG in the milk, the reverse was true in the serum of mothers where titers of IgA ( $35 \pm 17$ ) were significantly lower than IgG titers ( $386 \pm 75$ ; Wilcoxon matched pairs test,  $Z=2.20$ ;  $P=0.0277$ ) (Fig. 4B). Regarding the serum of litters, titers of IgA and IgG surpassed 1:100 dilution (Fig. 4C). If titers from pups are averaged per litter, IgA in the serum of litters ( $311.8 \pm 168.4$ ) reached higher titers than IgA in the serum of mothers ( $35.0 \pm 17.2$ ; Mann–Whitney  $U$ -test,  $Z_{\text{adj}}=2.14$ ;  $P=0.032$ ), but not so in the case of IgG (Mann–Whitney  $U$ -test,  $Z_{\text{adj}}=0.08$ ;  $P=0.935$ ). When globulins in the serum of litters were compared with those in the milk of mothers, titers of IgG in the pups were higher than those recorded in the mothers ( $26.0 \pm 6.0$ ; Mann–Whitney  $U$ -test,  $Z_{\text{adj}}=2.69$ ;  $P=0.007$ ), but not so in the case of IgA (Mann–Whitney  $U$ -test,  $Z_{\text{adj}}=0.43$ ;  $P=0.666$ ). More importantly, since IgA were not present in the fetuses examined (Section 3.2), IgA recorded in newborns must come from milk, implying that breeding females transfer specific antibodies to their offspring during lactation.

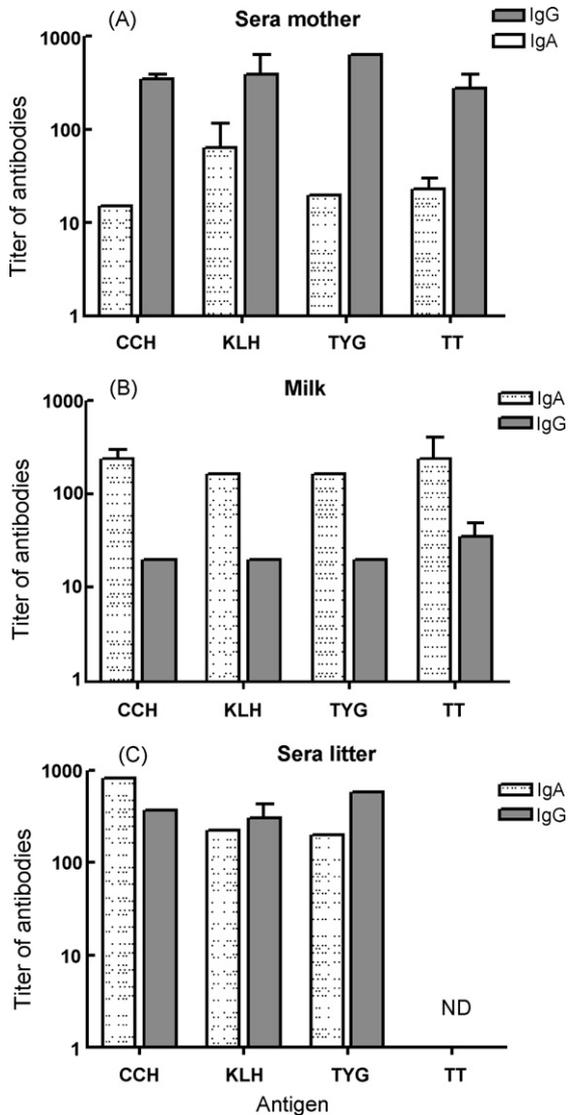


Fig. 4. Antibody transfer to offspring during lactation by *O. degus* mothers immunized with: loco (*C. concholepas*) hemocyanin (CCH without adjuvant;  $n=2$ ), keyhole limpet (*M. crenulata*) hemocyanin (KLH without adjuvant;  $n=1$ ), tetanus toxoid (TT with adjuvant,  $n=2$ ) and tyroglobulin (TYG with adjuvant;  $n=1$ ). Milk (A) and serum (B) of degu mothers were obtained within days 3–4 of lactation, and the presence of specific IgG and IgA isotypes against the antigens used to immunize each mother was determined through ELISA assays. (C) Serum from litters: CCH ( $n=6$  pups), KLH ( $n=6$  pups), TT ( $n=4$  pups) and TYG ( $n=5$  pups). Each bar represents the antibody titer in the milk or sera from mothers or litters  $\pm$  S.E. Bars without error bars denote single mothers or offspring sampled. The tetanus toxin could not be determined in the sera of litters due to a lack of reagents (ND).

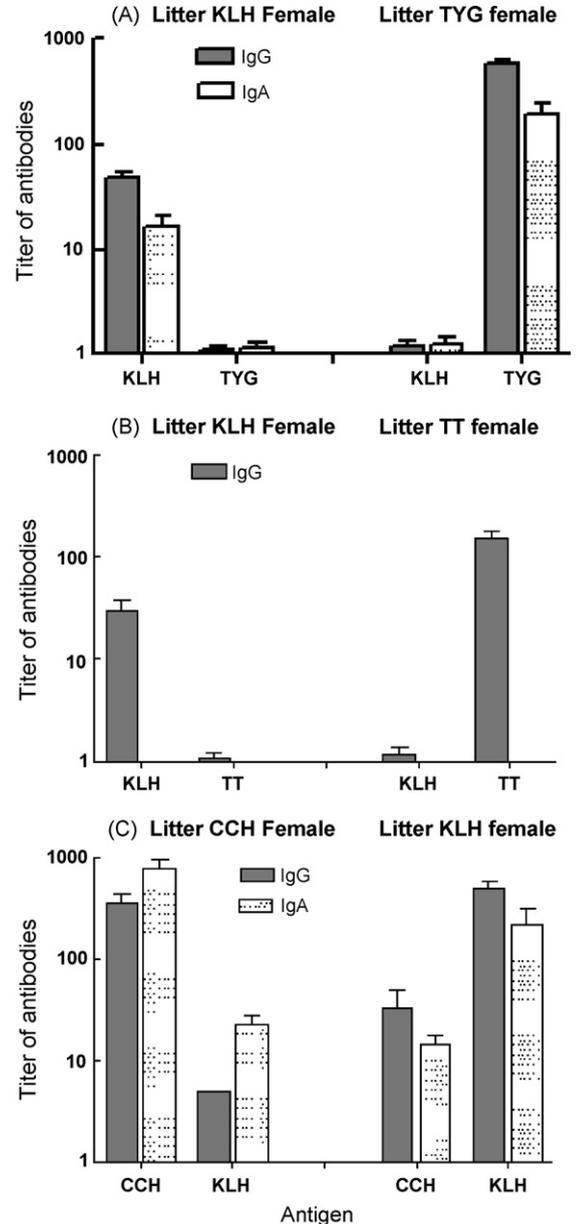


Fig. 5. Maternal antibody transfer of antibodies to unrelated offspring. Presence of IgG or IgA isotypes in the sera of communal litters whose mothers were previously immunized. (A) KLH-TYG communally breeding pair ( $n=9$  and 5 pups, respectively); (B) TT-KLH communally breeding pair ( $n=5$  and 8 pups, respectively) only IgG was tested; (C) KLH-CCH communally breeding pair ( $n=4$  and 6 pups, respectively). The presence of specific antibodies against homolog (i.e., that used to immunize the mother) and heterologous (i.e. antigen of the substitute mother) antigens were determined by indirect ELISA assays. Each bar represents mean antibody titers  $\pm$  S.E.

### 3.5. Maternal antibody transfer to unrelated offspring

We tested for evidence of allosuckling by offspring housed simultaneously with two lactating females. We used indirect ELISA essays to verify the presence of IgG and IgA in the sera of both litters, either against the antigen of their mother (homolog antigen) or the foster mother (heterologous antigen). We had three cases of communal breeding where two lactating females and their litters shared a cage. We recorded the presence of specific antibodies IgG and IgA isotypes against homolog antigen antibodies in the sera of litters in all three cases (Fig. 5A and C). In contrast, we detected the presence of specific IgG and IgA isotypes against heterologous antigens in the sera of litters in a single case, the pair CCH–KLH (Fig. 5C).

## 4. Discussion

Females of communally breeding rodents usually share a nest and rear their young together (Hayes, 2000; Lewis and Pusey, 1997; Solomon and Getz, 1997). Under these conditions, female breeders may incur a cost in providing milk and other parental resources to less related or totally unrelated offspring (Hayes, 2000). Since provision of parental care (including milk) to unrelated offspring is energetically costly (Thompson, 1992), and lactation has a negative impact on fitness of mothers in terms of subsequent survival and reproductive success (Clutton-Brock et al., 1989; Huber et al., 1999), communal breeding is expected to involve benefits that compensate such fitness costs. One hypothesized benefit includes that infants improve their immunocompetence through lactation from several females (Roulin and Heeb, 1999). Thus, suckling from two or more, genetically different, lactating females might provide newborns and young with a more diverse array of immunoglobulins and defensive cells.

One achievement of our study was to reveal that degus mothers could indeed transfer antibodies to their litters during pregnancy and lactation as well, a finding not previously reported in a wild communally breeding species. Future studies are needed to establish whether the transfer of antibodies during pregnancy in degus comes from the yolk sac, the placenta, or both (Ben-Hur et al., 2004). Similarly, the relative contributions of uterine and lactation stages during the transfer of IgG to litters need to be determined. In fact, the issue has not been addressed by previous studies on wild rodents documenting the generation of specific antibodies in response

to exotic antigens as probes of passive transfer from maternal immunoglobulins to offspring (Armitage and Gurri-Glass, 1994; Glass et al., 1990). Interestingly, IgA in mice and humans are abundant within the lumen of the gastrointestinal tract after milk ingestion, but not so in the circulation of newborns, implying that IgA are not transferred through the milk in these species. More recently, however, the transfer of significant quantities of IgA from milk into the circulation of the newborn has been demonstrated in foals and pigs (Sheoran et al., 2000; Harada et al., 2002; Elahi et al., 2006). Thus, degus add to recent findings suggesting that mothers may transfer their offspring with IgA during lactation in a social rodent.

Our study has provided limited support to the increased immunocompetence hypothesis where pups were expected to acquire antibodies from lactating females other than their mother. One out of three communal litters were detected to significantly exhibit antibodies not transferred from their mother. Moreover, some “transfer” of heterologous antibodies (Fig. 5C) may have resulted from cross-reaction between hemocyanins as these molecules share conserved amino acid sequences (Molledo et al., 2006; Oliva et al., 2002). A similarly low frequency of heterologous antibody titers was recorded in offspring of wild yellow-bellied marmot females inoculated with exotic albumin, myoglobin, thyroglobulin and KLH hemocyanins (Armitage and Gurri-Glass, 1994). Nonetheless, our inability to record transfer of heterologous antibodies in the offspring should be taken carefully. First, our study involved only a limited number of cases of communal breeding where two females were rearing their litters in a common nest. Second, our three cases of communal breeding were from female pairs whose litters were born with a time difference that ranged from 9 to 15 days. Thus, we cannot rule out that younger pups had limited opportunities to suckle from foster females. It is well known for rabbits that larger pups attain more milk, grow more and survive more than smaller pups (Bautista et al., 2005; Drummond et al., 2000). For degus, weanlings of litters born in the presence of a previous litter grow and survive less than weanlings born when no other young are present (Ebensperger et al., 2007), suggesting that younger pups suffer from competition with older, larger pups. Thus, more synchronized communal litters are needed, a difficult task in a rodent that breeds seasonally and whose reproductive biology is still poorly understood.

Alternatively, our data may reflect an overtly low probability of antibody transfer during degu lactation. The small intestine of newborn mammals is capable of

absorbing macromolecules (e.g., immunoglobulins) during a limited time period after birth, and this time period varies with species (Pácha, 2000). For instance, the transport capacity of newborn guinea pigs (a close relative of degus) decreases rapidly within the first day postpartum, while transport in hamsters ends after day 5 of lactation (Lecce and Broughton, 1973). Therefore, a time-lag in the ingestion of colostrum caused by birth asynchrony may result in offspring unable to attain antibodies from foster mothers, even though allosuckling takes place. Thus, studies assessing the dynamics of immunoglobulin uptake by newborn degus are strongly needed.

Establishing formal (theoretical) links between evolutionary ecology and immune function is a major goal in evolutionary biology (Grindstaff et al., 2003; Schmid-Hempel, 2003), and studies on the connection between social behavior and immune defense are particularly aimed at that goal (Roulin and Heeb, 1999; Traniello et al., 2002; Wilson et al., 2003). However, testing these ideas represents a major challenge, as details of model species' immune system need to be known precisely. While our long-term goal is to assess immunological benefits of communal breeding, the current study has demonstrated that antibodies can be transferred from mothers to their young from pregnancy through lactation in a wild social rodent. Given that antibodies persisted in young long enough to be detected, they could be used further to quantify the extent of communal nursing under more natural conditions (Armitage and Gurri-Glass, 1994).

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