

## SPLASH! milk science update: November 2013 issue



### SPLASH! milk science update NOVEMBER 2013 issue

This month's issue shares highlights from the 10th IMGC Symposium, addresses the complicated world of milk composition, tackles the issue of a "normal" infant breastfeeding pattern, and visits African cattle herds and their parasites. Enjoy!

## Top 10 of the 10th IMGC Symposium

The International Milk Genomics Consortium held its 10<sup>th</sup> annual conference on Human Health and Milk Genomics on October 1-3, 2013, at the University of California, Davis. The meeting included 29 talks, two panel discussions, a poster session, and a dinner. In case you missed it, here are the top ten moments.

**Number 10:** Dave Mills highlighted the potential for milk-based translational opportunities centered on developing a healthy microbiome. Specifically, in a crossover study with formula-fed and breastfed infants, his group showed that the beneficial gut bacteria *Bifidobacterium infantis* can only colonize well and persist in the presence of milk.

**Number 9:** Riveting panel discussions expounded upon daily presentations and future research applications of milk. Bruce German noted that modern cars have over 25 diagnostics running at all times monitoring the wellness of the vehicle, but humans have no similar external around-the-clock health gauge. The future of medicine will include non-invasive diagnostics, one of which may utilize compounds found in milk.

**Number 8:** Dining and musical festivities at the Old Sugar Mill reminded us that scientists may often be serious during the day, but we do like to let loose in good company. Exquisite cuisine, conga lines, and the Johan shuffle, as Bruce German kindly nicknamed it, were the highlights of this wonderful evening.

**Number 7:** Julie Sharp presented exciting findings of two newly discovered proteins exclusively appearing in monotreme lactation. These proteins are shown to have anti-bacterial properties, which likely evolved because the milk consumption environment of monotremes is not very sterile.

**Number 6:** The conference embraced social media to share the exciting research of the presenters with the public and fellow researchers. Highlights of Twitter postings by participants can be seen [here](#).

**Number 5:** Vengama Naidu Modepalli and Sanjana Kuruppath both investigate lactation in the tammar wallaby to learn about developmental advantages of marsupials, which help to increase their chances of survival despite early-gestation birth. Modepalli and Kuruppath found, respectively, wallaby milk supplementation to be effective for assisting the development of lung tissues and stomach tissues in two separate mouse models.

**Number 4:** Katie Hinde approaches milk through a specific evolutionary lens by investigating the influence of infant gender on the mother's milk composition. In a large-scale study, she found cows with daughters produce significantly more milk after their first parity. If optimized through the use of sex-selected semen, this could have vast implications for the dairy industry.



**Number 3:** Carlito Lebrilla shared some of his numerous contributions to glycomics that began fifteen years ago on a homebuilt FT-ICR mass spectrometer. His group has developed methods to quantify and classify the oligosaccharides in a vast number and type of samples. These methods can resolve molecular details like secretor status, species differences in milk, or even serve to phenotype gut microbiomes based on collective saccharide cleaving enzymes.

**Number 2:** Jeff Gordon delighted us with an outstanding body of research that highlighted the benefit of bovine milk oligosaccharides in the development of a healthy gut microbiome and their potential in food therapeutics for malnourished Malawi children.

**Number 1:** An increase in collaboration has been one of the biggest accomplishments over the ten years of the IMGC. Depicted as a web of connections, the IMGC began with a brave few and has now expanded into a network of informative collaborations and shared research objectives. Collaborations among IMGC scientists have increased 25-fold! Publications have increased by 1100%! Together, we have expanded the fields of milk biology, agriculture, and human health.

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## Mega Milk Composition Analysis

- **Skibieli and colleagues meta-analysis of 129 mammals' milks using statistical methods that account for phylogeny.**
- **Across mammals, milk composition reflects evolutionary history.**
- **Diet and lactation duration are important predictors of milk composition.**
- **Very dissimilar mammals can produce similar milks, but due to different selective pressures.**
- **To understand milk, we should study lactation strategies and life history.**



Fifty years ago Devorah Ben Shaul published the seminal paper "The Composition of the Milk of Wild Animals" (1963). She had spent ten years aggregating published papers of milk composition as well as directly analyzing dozens of species' milks. Eyeballing the data from 101 species, Ben Shaul posited that the composition of milks—the percent fat, protein, and sugar—did not necessarily cluster by the evolutionary history of taxonomic groups (a.k.a. phylogeny). She noted that "grizzly bear milk and kangaroo milk had virtually the same basic milk composition" (p. 333). Therefore, Ben Shaul approached milk from the perspective of environment and nursing behavior. She posited that milks clustered in relation to the degree of maturity at birth, maternal attentiveness and nursing frequency, and the exposure to water and ambient temperature. Mammals that park their infants and forage for extended periods of time, such as the echidna, rabbit, and lion, produce milk with high fat content to sustain infants until the next nursing bout. High fat milk also is typical in many seals and sea lions

in cold, aquatic environments because the pup's thermoregulatory needs are high, as is the need to develop a fat blubber layer. Species produced more dilute milk when young were "precocial," born at a relative mature condition, like ungulates, with eyes open, able to follow their mother on foraging trips from an early age, and with frequent access to suckling.

In subsequent decades, as more data both across and within species became available, two more very large reviews of milk composition were helmed by Olav Oftedal. He further considered milk composition through the lens of behavior and environment, as well as phylogeny (Oftedal, 1984; Oftedal & Iverson, 1995). Although milk composition was more likely to be similar among closely related species, behavioral care, environment, and other traits like body mass, also seemingly influenced milk synthesis. However, systematic statistical techniques for rigorously detecting “signatures” of phylogeny, environment, and behavior in milk composition across mammals remained absent from the literature.

Until now.

Presently in early view at the *Journal of Animal Ecology*, Amy Skibieli and colleagues combined data on mid-lactation milk composition from 129 mammalian species, representing 51 families and 15 different orders. For the first time, researchers have analyzed milk composition across monotremes, marsupials, and eutherians in relation to body mass, adaptation to arid environment, maternal diet, length of lactation, altricial versus precocial young, aquatic versus terrestrial habitats, and total reproductive output (litter size and neonate size).

Skibieli et al. (2013) used two different statistical approaches: one that did not account for phylogeny (ordinary least squares regression) and one that did (phylogenetic generalized least squares regression and regression with an Ornstein-Uhlenbeck transformation). Yeah, at first it seemed like gibberish to me, too. Basically, traditional regression models operate on the assumption that all data points are independent. BUT we know that milk synthesis has a genetic component and that closely related species will have vast similarities in their genomes. For this reason, two closely related species have a high probability of sharing many genes because they share a recent common ancestor. Regression analyses that compare species without accounting for phylogeny are violating assumptions that data points are independent. As a result, comparative analyses controlling for phylogeny are becoming increasingly important for understanding how selective pressures have shaped biological traits in animal and plant species (Nunn, 2011).

Skibieli and colleagues revealed that the “phylogenetic signal” in milk is very strong. Milk composition in a very large way reflects shared ancestry from a common ancestor. However, after controlling for phylogeny, duration of lactation and diet were both significantly associated with milk composition. Species that had longer periods of lactation produced more dilute milk—protracted lactation is seemingly not physiologically compatible with synthesizing higher energy density milk. This makes sense; mothers would too quickly become depleted if milk composition was energetically dense. Diet also had a strong influence on milk composition—carnivores synthesize milk higher in fat and protein (and consequently with higher dry matter content and caloric density (kcal/g)) than vegetarians or omnivores. Maternal body mass, arid environment, reproductive output (litter size and mass), and precociality were not associated with milk gross composition in this cross-mammal analysis (Skibieli et al., 2013) despite previous studies showing an association with some of these parameters within some clades (Hinde & Milligan, 2011). Mammals in aquatic environments produced richer milk after controlling for phylogeny, but this correlation was no longer important once diet and lactation length were included in statistical models. Importantly, the present analysis only investigated milk composition, but not milk yield. Total energy output, the combination of milk energy density and milk volume, is going to be a very critical component in understanding the effects of phylogeny, environment, and behavior (Riek, 2008).

So just like theoretical physicists, we still do not have a milk “Theory of Everything” because the mega mega analysis integrating all key data remains to be done. Milk synthesis exists at the complex intersection of the other dimensions of the lactation strategy: the duration of lactation, nursing pattern, and litter size. Skibieli and colleagues admirably tackled duration of lactation and total reproductive output (a measure related to litter size). But each of these dimensions involves many untested nuances, including the fact that the period of time between birth and weaning can involve substantial changes in milk composition across time as young transition from colostrum to exclusively milk feeding, to a period of mixed milk and solids feeding (Langer, 2008; Trott et al., 2002). Patterns of nursing—the frequency and duration of nursing bouts—can complicate milk throughput in the mammary gland, affecting milk composition (Miller et al., 2013). And litter size doesn’t account for the ratio of sons and daughters in the litter despite a growing literature suggesting that [infant sex may influence milk synthesis](#) (Hinde & Milligan, 2011). How mothers “pay” the costs of lactation may also have important interactions with milk synthesis. For most mammals, synthesizing milk reflects differential reliance on “capital” (the reliance on body stores) versus “income” (dietary intake) tactics to varying degrees across species and across individuals within species, and across time within an individual (Hinde et al., 2009; Jönsson, 1997). Also, across mammals the developmental priorities of young vary among growth, development, and behavioral activity budgets, so not only should nursing behavior be considered, but how infants are assimilating and utilizing the milk they ingest from their mother (Hinde, 2013). Natural selection is acting on adaptations for milk synthesis in the mother as well as adaptations in the infant to extract and use milk from the mother.

Yeah, it's a complex system. And for most of these parameters, reliable data are not yet available across the mammals for which we do have milk composition information, much less the thousands of other mammals whose milk composition we still haven't analyzed. Oh, and this is just talking about gross composition—the total percent fat, protein, and sugar—and doesn't even include description of the specific fatty and amino acids, or disaccharides and oligosaccharides, or the hundreds, possibly thousands, of other bioactive constituents in mammalian milk: hormones, minerals, immunofactors, etc. etc. etc. *ad nauseum*.

But even though we don't yet have the data available for the ONE ANALYSIS TO RULE THEM ALL!, we can be more confident about the things that we think we know. Milk reflects the immediate conditions of the mother and the environment as well as the conditions experienced by ancestors in the past. Species can have milk of the same composition as a result of different selective pressures. Conversely, species living in the same environment, with the same diet and same length of lactation, may still have very different milks because they evolved from different recent ancestors. Importantly, our fearless *SPLASH!* editor-in-chief, Danielle and colleagues, demonstrated that the genes underlying milk synthesis change at a slower rate than does other areas of the genome (Lemay et al., 2009), further complicating our studies of the evolution of mother's milk. Populations of a species may have recently expanded into a new environment, possibly leading to consumption of new types of food or tangling with new predators that require different vigilance and interactions with their young, all of which are likely to have important consequences for milk. In this way, milk synthesis reflects both the "here and now" AND the "there and then."

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## Defining Normal Breastfeeding Patterns

- **Breastfed infants control their milk intake to match their appetite and growth rate.**
- **Exclusively breastfed infants normally show a wide variety of breastfeeding patterns.**
- **Between one and three months of lactation, breastfed infants take fewer, faster, larger feeds, but their total daily milk intake is constant.**
- **Knowledge of the variability and expected changes in breastfeeding patterns can improve mothers' confidence about their milk supply.**

Parents need to have realistic expectations of their infant's breastfeeding behaviour to support the decision to breastfeed and avoid unnecessary supplementation. A recent publication on a longitudinal study of infant breastfeeding patterns and breastmilk intake provides much-needed evidence to give parents and health professionals confidence that there is a wide variation in normal breastfeeding behaviour, and also to demonstrate the changes parents can expect during the exclusive breastfeeding period.



Despite exclusive breastfeeding being the optimal way of feeding an infant, the percentage of mothers who exclusively breastfeed drops from over 75% who initiate to less than 11% at six months, well short of the Healthy People 2010 initiative goal of 50% of infants being exclusively breastfed for six months (Thulier and Mercer, 2009). The most frequent reason given by mothers for ceasing exclusive breastfeeding is a perception of insufficient milk (Hauck et al., 2011). Mothers' perceptions of their milk supply may be based on infant behaviour and whether that matches the expectations of the parents. If these expectations are based on the behaviour of formula-fed infants, then the expectations are likely to be unrealistic for a breastfed infant. If these expectations are not met, the mother may lose confidence in her breastfeeding. A loss of confidence is a major factor in the decision to cease breastfeeding or introduce supplementary feeds (Flores-Quijano et al., 2008).

Infant formula comes with instructions on how much and how often to feed an infant. Parents and caregivers may encourage an infant to finish each bottle of prepared formula. The formula does not contain leptin, which regulates food intake, while leptin is a component of breastmilk. The composition of formula thereby reduces the infant's self-regulation of milk intake. In addition, the rate of gastric emptying for formula is slower than for breastmilk, leaving the infant feeling full for longer and therefore potentially decreasing the frequency of feeding compared to a breastfed infant. Moreover, breastmilk has a lower casein concentration and forms soft, easily digestible curds in the stomach, so the infant may be ready to feed again after a short interval. For these reasons, infant feeding patterns may differ between breastfeeding infants and those receiving formula. If a breastfed infant cues to feed again after a short interval, the mother may assume that her infant has not received enough breastmilk during the previous breastfeed and mistakenly perceive that she has insufficient breastmilk.

Parents need to know that breastfed infants have a wide range of feeding behaviour. This has been documented in a cross-sectional study of exclusively breastfed infants between one and six months old, published in *Pediatrics* (Kent et al., 2006). The data dispel any belief that breastfed infants follow the same patterns of breastfeeding behaviour as formula-fed infants. The breastfed infants, who were all growing normally, cued to feed between four and 13 times a day. Some of the infants (13%) only ever fed from one breast during each breastfeeding session, while 30% of the infants always fed from both breasts during each breastfeeding session. The remainder sometimes fed from one breast, sometimes fed from both breasts, and occasionally had a 'cluster feed', going back to the first breast after finishing feeding on the second breast. For a 24-hour period, mothers measured the milk intake of their infants by weighing their infants before and after each breastfeed, referred to as test-weighing. These data showed that rather than taking the same amount during each breastfeeding session, the infants consumed between 0 mL (the infant went to the breast and apparently suckled but removed no milk) and 240 mL. The infants who breastfed less frequently consumed more milk during each breastfeeding session than the infants who fed more frequently. However, this relationship does not result in all infants receiving the same amount of breastmilk. There is a wide range in the total amount of breastmilk consumed by normal, exclusively breastfeeding infants, ranging from 478 to 1356 mL. The data show that the total milk intake is independent of the feeding frequency.

Measurement of the fat content of milk samples collected from the mother before and after each test-weighing shows that breastfed infants feed to appetite and do not always 'empty the container'. The fat content of the samples allowed the researchers to calculate the amount of milk available in the breast and the proportion of the available milk that was removed from each breast during each breastfeed. They determined that the infants removed, on average, only 67% of the available milk and normally only drained the breast once a day. In addition, they ascertained that the total daily fat intake of the infant ranged from 15 to 50 g. and was also independent of the breastfeeding frequency.

The results of this cross-sectional study serve to inform parents and health professionals that there is a wide range in normal breastfeeding behaviour and breastmilk intake and that breastfed infants do not need to conform to an average or to the feeding behaviour and intake of bottle-fed infants. However, mothers may be concerned if their infants breastfeed less often or more quickly than usual, or if their breasts feel softer and less full as lactation progresses.

The longitudinal study of breastfeeding behaviour published recently in *Breastfeeding Medicine* by the Hartmann Human Lactation Research Group (Kent et al., 2013) recorded breastfeeding patterns and milk intake for each breastfeeding session over two to five 24-hour periods between one month and six months of lactation. This study has provided the necessary evidence to show that at every age there is a wide range in the number of breastfeeds in a day, but on average the frequency decreases between one and three months, after which it stabilises.

There is also a wide range in the duration of each breastfeeding session at every age, but on average the duration decreases between one and six months. However, rest assured that the infant is simply becoming more efficient at suckling; the amount of breastmilk consumed during each feeding increases between one and three months. The result is that, for each infant, the total amount of breastmilk consumed remains constant during exclusive breastfeeding between one and six months. Manufacturers of infant formula might consider taking this information into consideration and revising their feeding tables.

It is normal for breastfeeding infants to feed at night, even at six months of age. However, Kent et al. show that while one-month-old infants need frequent feeding day and night, the longest interval between feedings increases between one and three months. This may be related to the demonstrated increase in the maximum milk intake during a feeding, which may be a reflection of the increasing stomach capacity of the infant.

Both of these studies will help to reassure parents that changes in their infant's breastfeeding behaviour are normal and not an indication of insufficient milk supply. Instead, objective clinical indicators, such as infant weight gain based on the WHO's breastfed infant growth charts, should be used to determine whether to supplement with infant formula.

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## Identifying Parasites in African Cattle

- **Scientists in Vienna, Austria, have developed a diagnostic that simultaneously tests for three species of cattle-infecting trypanosome parasite.**
- **The test should help in monitoring the parasites' distribution in Africa, and ultimately reduce the impact on milk (and meat) production, thus improving local farmers' incomes.**
- **The researchers' subsequent survey of infection rates across Burkina Faso highlights the importance of conserving local cattle breeds, which have been selected for their partial immunity to trypanosomes.**

When African cattle become lazy and their milk production drops, they are said to have 'nagana'—to be depressed, in Zulu. The biological cause of nagana is a parasite, of which there are several species, and which wiggle like winding corkscrews under a microscope. To work out how to reduce the damage done by these 'trypanosome' parasites, and thus improve the lot of many poor cattle farmers, we need better diagnostics. Recently, a group of researchers in Vienna invented a means of testing for three different species of trypanosome at the same time.

What explains the usefulness of their work is the fact that many trypanosome species have overlapping geographical distributions. And the appropriate treatment for infected cattle hinges on the type of trypanosome that is causing the symptoms.

Older diagnostics can pick up the presence of trypanosomes, but can't pick *apart* the different species. The tests involve, for example, examining a blood sample under a microscope for the parasites' typical turning motion, or spotting the little critters among white blood cells, after a blood sample has been whirled in a centrifuge.

Even though different trypanosome species may look the same, their genomes must be distinguishable, provided you know how to read them. The new test, created by Katja Sibermayr, of the University of Veterinary Medicine in Vienna, Austria, and her colleagues, analyses trypanosome DNA. It employs a technology called quantitative PCR, which quickly shows a positive result if a blood sample contains certain DNA sequences that are unique to each of three trypanosome species—*Trypanosoma congolense*, *T. brucei*, and *T. vivax*. The test indicates which species caused the DNA match. "PCR-based methods are quite difficult to perform in the field because they require sophisticated equipment," explains Sibermayr. "[But] I think that our novel qPCR assay will be used in centralized trypanosome monitoring and surveillance facilities."

All three of these trypanosome species occur in Burkina Faso, where Sibermayr's team put the fruits of their labor into practice. They collected blood samples from cattle—from Zebu and Baoulé breeds, plus hybrids of the two—from 72 farms in 24 villages.

These days many African cattle breeds face potential extinction. The results of Sibermayr et al's survey therefore read like a fable, warning of the pressing need for genetic conservation. Less than 10% of the Baoulés, a local West African dwarf breed, and of Zebu-Baoulé hybrids were infected. Those that were lost weight and developed a fever, but that was the extent of their plight.

As expected, the Zebus, a breed that was introduced from South Asia for its high milk yields and ability to pull heavy ploughs, were most likely to harbor trypanosomes. About 22% of Zebus tested were infected. Moreover, their health was badly damaged by infection. "Baoulés seem able to tolerate higher amounts of the parasite," says Sibermayr. "We only studied [relatively] healthy animals, and Zebus with such high parasite levels would have been too ill to be included in our study." The new diagnostic was also able to pick up cases where a single cow was infected with more than one species of trypanosome.



Zebu (*Bos indicus*)

The genetic basis of the Baoulés' and the crossbred cattle's partial immunity is not clear. But the case for greater effort to protect such an important genetic source certainly is: without a stock of pure-bred Baoulés, farmers would not be able to keep mating them with Zebus to create the crossbreeds endowed with Zebu-like milk yields and Baoulé-like robustness towards nagana. That kind of effort will likely require international funding.

For now, though, this new diagnostic test, aside from guiding how infected animals would be best treated, provides a welcome insight into the spread of parasites that do their part in sustaining poverty cycles.

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