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A possible role of social activity to explain differences in publication output among ecologists

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Publication output is the standard by which scientific productivity is evaluated. Despite a plethora of papers on the issue of publication and citation biases, no study has so far considered a possible effect of social activities on publication output. One of the most frequent social activities in the world is drinking alcohol. In Europe, most alcohol is consumed as beer and, based on well known negative effects of alcohol consumption on cognitive performance, I predicted negative correlations between beer consumption and several measures of scientific performance. Using a survey from the Czech Republic, that has the highest per capita beer consumption rate in the world, I show that increasing per capita beer consumption is associated with lower numbers of papers, total citations, and citations per paper (a surrogate measure of paper quality). In addition I found the same predicted trends in comparison of two separate geographic areas within the Czech Republic that are also known to differ in beer consumption rates. These correlations are consistent with the possibility that leisure time social activities might influence the quality and quantity of scientific work and may be potential sources of publication and citation biases.

Publication success (i.e. the number and citation rate of scientific papers) is a widely used surrogate metric in assessments of the academic performance at all levels from individual scientists to national reviews (Cassey and Blackburn 2004, Leimu and Koricheva 2005). Both publication and citation success were previously shown to be influenced by variety of factors, including statistical (non)significance of results, number of co-authors, last name of co-authors, nationality and scientific field of enquiry (Møller and Jennions 2002, Tregenza 2002, Leimu and Koricheva 2005, Wong and Kokko 2005). However, to my knowledge no study has to date investigated external factors less tightly connected to the publication process itself, e.g. social and recreational activities (cf. Fig. 2 in Lortie et al. 2007).

One of the most common social activities in the world is alcohol consumption – estimated "2 billion people worldwide consume alcoholic beverages" (World Health Organization 2004, p. 1). In Europe, this is mostly in the form of beer drinking (World Health Organization 2004, Table 4). I chose to test the effect of alcohol consumption on scientific performance because this hypothesis leads to unequivocal predictions. This is because it is well known that alcoholic drinks negatively affect human cognitive capabilities that are critical for any physical and mental performance not to speak of such a demanding activity as producing a high quality science. Specifically, alcoholic beverages, including beer, impair "memory, abstracting, problem-solving, perceptual analysis and synthesis, speed of information processing, and efficiency" (Parsons 1998, p. 954). Thus, human cognitive performance during and after drinking is decreased at least in the short term and costs of beer drinking extend beyond the mere time spent with this activity. This does not specifically hold for other recreational activities. Moreover, potential effects on scientific productivity of other such activities, e.g. mountain climbing or picking mushrooms (Røskaft et al. 2004), are unclear and hard to predict. Further, alcohol consumption is positively associated with depressive symptoms (Palfai et al. 2007) and depression, with symptomatic loss of motivation, adversely affects work productivity (Stewart et al. 2003). Moreover, cooperativeness is critical for successful performance in science (Leimu and Koricheva 2005) and alcohol was shown to significantly decrease cooperativeness within groups (Hopthrow et al. 2007). Alcohol drinking also leads to work alienation (Greenberg and Grunberg 1995). Thus, both short-term direct (physiological) and long-term indirect (psychological) effects of alcohol use are well known to decrease mental and working performance in general. Here I predict the same negative effects for scientific performance in particular. The Czech Republic, with traditionally the highest consumption of beer per capita in the world (156.9 litres per year; Kirin Research Institute of Drinking and

COMMENT

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The lost correspondence of Francis Crick

Alexander Gann and **Jan Witkowski** unveil newly found letters between key players in the DNA story. Strained relationships and vivid personalities leap off the pages.

In the summer of 1975, James Watson wrote to Francis Crick proposing that something be published on the story of the RNA Tie Club, an informal group of 24 members who exchanged ideas about RNA and the genetic code. Crick responded, on 16 July: "Almost all my own early correspondence was unfortunately thrown away without my knowledge by an over-efficient secretary." The Wellcome Library in London, which acquired the majority of Crick's professional papers from him in 2001, also quote this passage and warn that: "Researchers should note that there has been some loss of early correspondence."

It turns out that this lost correspondence was never thrown out, but became mixed in with Sydney Brenner's papers. Brenner and Crick shared an office in Cambridge from 1956 to 1977. They moved offices and buildings several times — from the Cavendish Laboratory to the 'Hut' to the new Medical Research Council (MRC) Laboratory of Molecular Biology (LMB), and between offices within the LMB. It is not surprising that some of Crick's correspondence became intermingled with Brenner's papers. A line in a 1961 letter from Crick to the eminent phage geneticist Waclaw Szybalski supports this conjecture: "Do forgive me for not replying earlier to your letter of 15th December, but it arrived at Christmas time and got mislaid among Sydney Brenner's papers."

Earlier this year, we found the missing correspondence in the papers that Brenner donated to the Cold Spring Harbor Laboratory Library archives. The extensive Crick material, nine archive boxes of correspondence, photographs, postcards, preprints, reprints, meeting programmes, notes and newspaper cuttings, dates from 1950 to 1976, the bulk from the mid-1950s to the mid-1960s. (The catalogue of the complete Brenner Collection is at go.nature.com/6mYBhP.)

The letters of greatest interest, unveiled here for the first time, are those between Crick and Maurice Wilkins when they were both searching for the structure of DNA. They reveal telling details of the relations between the rival parties, and give vivid insights into the personalities involved. There is also previously unknown correspondence to and from other key players in the development of molecular biology.

COLD SPRING HARBOR LABORATORY ARCHIVES

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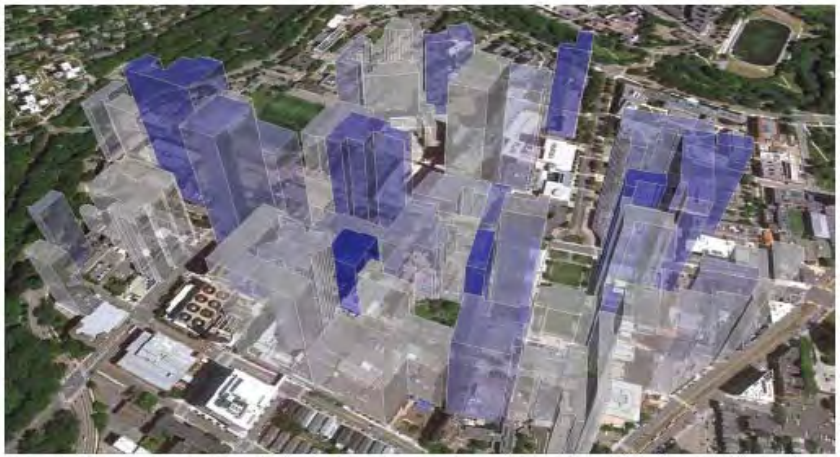

A schematic of the Longwood campus of Harvard Medical School shows the mean number of publication citations originating from each building (height), and the proportion of publications in each building where first and last authors work (grey is low, blue is high). Statistically, bluer buildings are also higher.

BIBLIOMETRICS

Love thy lab neighbour

Getting closer to your collaborators boosts a paper's citations.

BY RICHARD VAN NOORDEN

Anyone who has worked in a laboratory probably feels that having key members of the group placed closer together makes for a better research project. A study linking the proximity of investigators and the impact of their research now backs up that hunch.

Isaac Kohane, co-director of the Harvard Medical School Center for Biomedical Informatics in Boston, Massachusetts, decided to put intuition to the test in 2005 after a debate with Harvard's dean of administration, Richard Mills, over the layout of the centre. "I felt this viscerally, but there was no hard evidence," says Kohane. He enlisted more than a dozen undergraduates to identify 35,000 articles published between 1999 and 2003 in biomedical sciences, each with at least one Harvard author. It took the team two years to pinpoint where individual Harvard investigators were working — right down to the level of individual offices and laboratories.

The results, published in *PLoS ONE* last week (K. Lee *et al. PLoS ONE* 5, e14279; 2010), show that the shorter the geographical distance between first and last authors on a paper, the more highly cited were their research papers. First authors often bear the brunt of the work, whereas last authors tend to take the lead organizational role — and both are key players in the research project. The distance trend was not found for middle authors, who could be far removed from other collaborators without any clear effect on research impact.

Kohane and his colleagues also looked at individual buildings on the four campuses across which Harvard life-science research happens to be spread. They found that the more that researchers within a building tended to collaborate with one another rather than with people elsewhere, the more highly cited the publications that came from that building (see picture). The team does acknowledge an alternative explanation for the data: that scientists might choose to keep potentially high-impact breakthroughs within their own laboratory, or within a close circle of researchers.

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This seems to be the first empirical study of the connection between proximity and impact, says Anthony van Raan, an expert in using citation analyses to study scientific productivity and impact at Leiden University, the Netherlands. Most studies of the relationship between spatial separation and scientific impact have been done on a national and international scale, for which it has been demonstrated many times that international collaborations produce more highly cited science than local collaborations — probably a consequence of the size and scope of such efforts.

Kohane speculates that international collaborations might become even more successful if the first and last authors worked very close together, something that has not yet been tested. He certainly practises what he preaches: he and first author Kyungjoon (Joon) Lee, who coordinated the undergraduates' fact-finding, now work on the same floor. "When the study started we were on different floors," says Kohane, "and Joon told me that I became a lot more helpful when I moved to his floor." ■

+ K. Lee et al. in PLOS one 5

LETTER

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Reliability of flipper-banded penguins as indicators of climate change

Claire Saraux[1,2,3,4], Céline Le Bohec[1,2,3], Joël M. Durant[3], Vincent A. Viblanc[1,2], Michel Gauthier-Clerc[5], David Beaune[1,2], Young-Hyang Park[6], Nigel G. Yoccoz[7], Nils C. Stenseth[3,8] & Yvon Le Maho[1,2]

In 2007, the Intergovernmental Panel on Climate Change highlighted an urgent need to assess the responses of marine ecosystems to climate change[1]. Because they lie in a high-latitude region, the Southern Ocean ecosystems are expected to be strongly affected by global warming. Using top predators of this highly productive ocean[2] (such as penguins) as integrative indicators may help us assess the impacts of climate change on marine ecosystems[3,4]. Yet most available information on penguin population dynamics is based on the controversial use of flipper banding. Although some reports have found the effects of flipper bands to be deleterious[5–8], some short-term (one-year) studies have concluded otherwise[9–11], resulting in the continuation of extensive banding schemes and the use of data sets thus collected to predict climate impact on natural populations[12,13]. Here we show that banding of free-ranging king penguins (*Aptenodytes patagonicus*) impairs both survival and reproduction, ultimately affecting population growth rate. Over the course of a 10-year longitudinal study, banded birds produced 39% fewer chicks and had a survival rate 16% lower than non-banded birds, demonstrating a massive long-term impact of banding and thus refuting the assumption that birds will ultimately adapt to being banded[6,12]. Indeed, banded birds still arrived later for breeding at the study site and had longer foraging trips even after 10 years. One of our major findings is that responses of flipper-banded penguins to climate variability (that is, changes in sea surface temperature and in the Southern Oscillation index) differ from those of non-banded birds. We show that only long-term investigations may allow an evaluation of the impact of flipper bands and that every major life-history trait can be affected, calling into question the banding schemes still going on. In addition, our understanding of the effects of climate change on marine ecosystems based on flipper-band data should be reconsidered.

The effects of climate forcing on primary and secondary production of the short austral food webs may be integrated at higher levels[14,15], and thus amplified in top-level predators such as seabirds. This has led to a strong interest in studying Antarctic and sub-Antarctic top predators (especially penguins, which are major consumers of the Southern Ocean ecosystem) as sensitive indicators of environmental changes[3,4]. To understand how variability in marine resources affects their demography over the timescale of years, simultaneous investigations of variation in breeding success and survival are necessary and require long-term individual monitoring at the population scale.

Most of our present knowledge on the population dynamics of penguins is based on large flipper-banding schemes. The key advantage is that bands can be identified from a distance, avoiding recapture stress for the birds. In the 1970s, however, bands were observed, both in zoos and in the wild, to injure flipper tissues severely[5], especially during the moult. Although many research programmes consequently abandoned banding as a precaution in the late 1980s, massive banding schemes still continued (see references in ref. 5). Yet, as penguins power their swimming exclusively with their flippers, there has been an increasing concern about the hydrodynamic drag effect that may be induced by flipper bands (for example a 24% increase in the energy cost of swimming in captive Adélie penguins[8]). The question was then whether penguins may compensate for such effects[10,11] and whether the impact of flipper bands would be limited in time. Although it had been assumed that the effect of flipper bands lasted for a year at most[12] (until the bird got used to the band), the question remained to be addressed in the long term. In this context, medium-term studies revealed lower breeding success and survival in Adélie penguins[8] and a reduced breeding success in king penguins[7]. However, those pioneering findings did not result in the cessation of ongoing banding schemes. Whether or not flipper bands have a deleterious impact in the long term is, nonetheless, a crucial issue, for "it raises practical and larger ethical questions about costs and benefits of procedures in field studies"[16]. In addition to possibly harming penguin populations already under threat (such as penguins rehabilitated after oil spills; see references in ref. 7), the potentially negative effects of banding on demographic parameters may introduce a bias, which in turn might jeopardize any attempt to use data from banded birds to assess the impact of climate[12] on population dynamics and to predict the future

Table 1 | Observed differences between life-history traits of banded and non-banded king penguins

Parameter	Non-banded	Banded	*P* (banding)
Early group			
Arrival dates	21 Nov. ± 2 d (189)	7 Dec. ± 3 d (167)	<0.001
Breeding propensity	0.95 (189)	0.87 (167)	0.04
Laying dates	29 Nov. ± 1 d (160)	6 Dec. ± 1 d (122)	<0.001
Breeding success	0.44 (160)	0.32 (122)	0.05
Foraging trips	11.60 ± 0.20 d (512)	12.70 ± 0.20 d (344)	<0.001
All birds			
Overall survival over the decade	0.36	0.20	0.04
Overall/annual survival over the first period (4.5 years)	0.62/0.90	0.32/0.78	0.01
Overall/annual survival over the last period (5.5 years)	0.57/0.90	0.60/0.91	0.82

Significant results are indicated in bold. Data shows mean ± s.e. The number of events (*N*) is shown in parentheses. Differences in *N* come from differences in studied stages (for example, not every bird arriving at the colony bred). Overall survival corresponds to the number of studied birds present at the colony at the end of the period divided by the number of studied birds present at the colony at the beginning of the period. Breeding propensity corresponds to the proportion of live birds that engaged in reproduction over the ten breeding seasons (that is, the number of reproduction events divided by the sum over the years of live birds). The early group in the group of birds that failed or did not engage in previous reproduction and were free to arrive early in the summer. This group is the one that most affects overall population reproductive success. For breeding analyses, *P* is the *P* value of the banding effect in the mixed model Y = banding + (1 | individual). For survival (investigated through schemes of presence/absence at the colony), *P* is the *P* value obtained from a Cox proportional hazards model with right censoring. Durations of foraging trips were standardized per period and then pooled together to run a single mixed-model analysis.

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ARTICLE

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Genetic history of an archaic hominin group from Denisova Cave in Siberia

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Using DNA extracted from a finger bone found in Denisova Cave in southern Siberia, we have sequenced the genome of an archaic hominin to about 1.9-fold coverage. This individual is from a group that shares a common origin with Neanderthals. This population was not involved in the putative gene flow from Neanderthals into Eurasians; however, the data suggest that it contributed 4–6% of its genetic material to the genomes of present-day Melanesians. We designate this hominin population 'Denisovans' and suggest that it may have been widespread in Asia during the Late Pleistocene epoch. A tooth found in Denisova Cave carries a mitochondrial genome highly similar to that of the finger bone. This tooth shares no derived morphological features with Neanderthals or modern humans, further indicating that Denisovans have an evolutionary history distinct from Neanderthals and modern humans.

Less than 200,000 years ago, anatomically modern humans (that is, humans with skeletons similar to those of present-day humans) appeared in Africa. At that time, as well as later when modern humans appeared in Eurasia, other 'archaic' hominins were already present in Eurasia. In Europe and western Asia, hominins defined as Neanderthals on the basis of their skeletal morphology lived from at least 230,000 years ago before disappearing from the fossil record about 30,000 years ago[1]. In eastern Asia, no consensus exists about which groups were present. For example, in China, some have emphasized morphological affinities between Neanderthals and the specimen of Maba[2], or between *Homo heidelbergensis* and the Dali skull[3]. However, others classify these specimens as 'early *Homo sapiens*'[4]. In addition, until at least 17,000 years ago, *Homo floresiensis*, a short-statured hominin that seems to represent an early divergence from the lineage leading to present-day humans[5–7], was present on the island of Flores in Indonesia and possibly elsewhere.

DNA sequences retrieved from hominin remains offer an approach complementary to morphology for understanding hominin relationships. For Neanderthals, the nuclear genome was recently determined to about 1.3-fold coverage[8]. This revealed that Neanderthal DNA sequences and those of present-day humans share common ancestors on average about 800,000 years ago and that the population split of Neanderthal and modern human ancestors occurred 270,000–440,000 years ago. It also showed that Neanderthals shared more genetic variants with present-day humans in Eurasia than with present-day humans in sub-Saharan Africa, indicating that gene flow from Neanderthals into the ancestors of non-Africans occurred to an extent that 1–4% of the genomes of people outside Africa are derived from Neanderthals[8]. In addition, ten partial and six complete mitochondrial (mt)DNA sequences have been determined from Neanderthals[9–17]. This has shown that all Neanderthals studied so far share a common mtDNA ancestor on the order of 100,000 years ago[10], and in turn, share a common ancestor with the mtDNAs of present-day humans about 500,000 years ago[10,16,18] (as expected, this is older than the Neanderthal–modern human population split time of 270,000–440,000 years ago estimated from the nuclear genome[8]). One of these mtDNA sequences has also shown that hominins carrying mtDNAs typical of Neanderthals were present as far east as the Altai Mountains in southern Siberia[13].

In 2008, the distal manual phalanx of a juvenile hominin was excavated at Denisova Cave. This site is located in the Altai Mountains in southern Siberia, and is a reference site for the Middle to Upper Palaeolithic of the region where systematic excavations over the past 25 years have uncovered cultural layers indicating that human occupation at the site started up to 280,000 years ago[20]. The phalanx was found in layer 11, which has been dated to 50,000 to 30,000 years ago. This layer contains microblades and body ornaments of polished stone typical of the 'Upper Palaeolithic industry' generally thought to be associated with modern humans, but also stone tools that are more characteristic of the earlier Middle Palaeolithic, such as side-scrapers and Levallois blanks[21–23].

Recently, we used a DNA capture approach[10] in combination with high-throughput sequencing to determine a complete mtDNA genome from the Denisova phalanx. Surprisingly, this mtDNA diverged from the common lineage leading to modern human and Neanderthal mtDNAs about one million years ago[19], that is, about twice as far back in time as the divergence between Neanderthal and modern human mtDNAs. However, mtDNA is maternally inherited as a single unit

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LETTERS

The complete mitochondrial DNA genome of an unknown hominin from southern Siberia

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With the exception of Neanderthals, from which DNA sequences of numerous individuals have now been determined[1], the number and genetic relationships of other hominin lineages are largely unknown. Here we report a complete mitochondrial (mt) DNA sequence retrieved from a bone excavated in 2008 in Denisova Cave in the Altai Mountains in southern Siberia. It represents a hitherto unknown type of hominin mtDNA that shares a common ancestor with anatomically modern human and Neanderthal mtDNAs about 1.0 million years ago. This indicates that it derives from a hominin migration out of Africa distinct from that of the ancestors of Neanderthals and of modern humans. The stratigraphy of the cave where the bone was found suggests that the Denisova hominin lived close in time and space with Neanderthals as well as with modern humans[2–4].

The first hominin group to leave Africa was *Homo erectus* about 1.9 million years (Myr) ago[5]. Archaeological as well as genetic data indicate that at least two groups of hominins left Africa after this event: first, the ancestors of the Neanderthals between 500,000 and 300,000 years ago (500 and 300 kyr ago, respectively), presumably *Homo heidelbergensis* or *Homo rhodesiensis*[6–9]; and, second, anatomically modern humans about 50 kyr ago[10,11]. Despite recent advances in the retrieval of ancient DNA from Neanderthals and early modern humans[12–14], DNA sequences have not been recovered from other Pleistocene hominins such as *H. erectus*, *H. heidelbergensis* or *Homo antecessor*[15]. A major reason for this is that exceptional circumstances are required for DNA to survive over long time periods. DNA degradation increases with temperature and soil conditions such as acidity[16], and most early hominin fossils come from equatorial and tropical regions in Africa and Eurasia, where conditions for DNA survival are therefore poor. Thus, although direct descendants of *H. erectus* might have survived until less than 100 kyr ago in Indonesia[17], it is unlikely that endogenous DNA will be retrieved from these hominins. However, archaeological evidence suggests that archaic hominins such as *H. erectus*, *H. heidelbergensis* and Neanderthals also lived at higher latitudes where the potential for DNA preservation is better. One such region is the Altai Mountains in southern Siberia[4] where hominin occupation may go back to more than 125 kyr ago (for example, the Karama site)[18].

Fossils complete enough for morphological classification do not exist from most sites in the Altai. Rather, small pieces of human skeletons such as teeth and bone fragments are generally recovered[4,19]. In 2008, the distal manual phalanx of the fifth digit of a hominin was excavated in Denisova Cave (51° 40′ N; 84° 68′ E) in the Altai Mountains, Russia. Episodic hominin occupation has been documented at this site for at least 125 kyr[20] and the phalanx was found in a stratum (layer 11) dated to 48–30 kyr ago where an assemblage containing both Upper and Middle Palaeolithic elements has been reported (see Supplementary Information).

We extracted DNA from 30 mg of bone powder and converted it into an Illumina sequencing library using DNA adaptors that carry project-specific barcodes. We next used a recently published protocol for targeted sequence retrieval called primer extension capture (PEC)[1] to isolate mtDNA fragments from the entire mitochondrial genome. The isolated fragments were sequenced from both ends on the Illumina GA$_{II}$ platform, using 76 cycles for each read. Fragments were included in further analyses if their forward and reverse reads overlapped by at least 11 base pairs (bp) and thus could be merged into single sequences. This removes all fragments over ~134 bp from analysis, but reduces errors at the 3′ ends of Illumina reads where error rates are highest[21,22]. We generated 1,178,300 merged sequences, of which 93,349 (7.9%) aligned to the revised Cambridge Reference Sequence (rCRS)[23] using an iterative mapping assembler, MIA[1]. This alignment program is particularly suitable for aligning ancient DNA sequences because it takes into account the frequent sequence errors associated with base damage in ancient DNA sequences[24,25]. Owing to the library amplification steps involved in the primer extension capture procedure, multiple copies of each original DNA molecule may be sequenced. Thus, fragments with identical start and end coordinates were merged to single sequences where at each position the base with the highest quality score was used. A total of 30,443 such distinct sequences were then used to assemble a mtDNA sequence[1]. The final assembled mtDNA sequence was identical irrespective of whether the reference mtDNA sequence used for the assembly was modern human or Neanderthal. Coverage across the mtDNA was high (mean = 156-fold, lowest 2-fold, highest 602-fold; Supplementary Fig. 1).

Several different approaches were used to assess the reliability of the mtDNA sequence determined. First, we made a second DNA extract from the Denisova phalanx and determined 9,908 mtDNA fragments by shotgun sequencing on the Illumina platform. From the reads we assembled a complete mtDNA sequence that was identical to the one retrieved with the PEC approach (Supplementary Information). This shows that the mtDNA sequence retrieved is reproducible and not dependent on the particular primers used for PEC. Second, we used 276 sequence positions where the Denisova hominin mtDNA is different from >99% of present-day human mtDNAs to assess whether the mtDNA recovered with PEC comes from a single individual. Among the 15,008 fragments that overlap these 276 positions, 14,961 carry the bases of the Denisova hominin mtDNA. This suggests that 99.7% (95% confidence interval; 99.6–99.8%) of all mtDNA fragments in the bone come from a single individual. Third, we used three physical features of the DNA fragments sequenced to gauge their extent and patterns of degradation: (1) the average length of the

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SABOTAGE!

Postdoc Vipul Bhrigu destroyed a colleague's experiments to get ahead. It took a hidden camera to expose a little-known, malicious side of science.

BY BRENDAN MAHER

It is sentencing day at Washtenaw County Courthouse, a drab structure of stained grey stone and tinted glass a few blocks from the main campus of the University of Michigan in Ann Arbor. Judge Elizabeth Pollard Hines has doled out probation and fines for drunk and disorderly conduct, shoplifting and other mundane crimes on this warm July morning. But one case, number 10-0596, is still waiting. Vipul Bhrigu, a former postdoc at the university's Comprehensive Cancer Center, wears a dark-blue three-buttoned suit and a pinched expression as he cups his pregnant wife's hand in both of his. When Pollard Hines calls Bhrigu's case to order, she has stern words for him: "I was inclined to send you to jail when I came out here this morning."

Bhrigu, over the course of several months at Michigan, had meticulously and systematically sabotaged the work of Heather Ames, a graduate student in his lab, by tampering with her experiments and poisoning her cell-culture media. Captured on hidden camera, Bhrigu confessed to university police in April and pleaded guilty to malicious destruction of personal property, a misdemeanour that apparently usually involves cars: in the spaces for make and model on the police report, the arresting

Genomic Comparison of the Ants *Camponotus floridanus* and *Harpegnathos saltator*

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The organized societies of ants include short-lived worker castes displaying specialized behavior and morphology and long-lived queens dedicated to reproduction. We sequenced and compared the genomes of two socially divergent ant species: *Camponotus floridanus* and *Harpegnathos saltator*. Both genomes contained high amounts of CpG, despite the presence of DNA methylation, which in non-Hymenoptera correlates with CpG depletion. Comparison of gene expression in different castes identified up-regulation of telomerase and sirtuin deacetylases in longer-lived *H. saltator* reproductives, caste-specific expression of microRNAs and SMYD histone methyltransferases, and differential regulation of genes implicated in neuronal function and chemical communication. Our findings provide clues on the molecular differences between castes in these two ants and establish a new experimental model to study epigenetics in aging and behavior.

As eusocial insects, ants live in populous colonies in which up to millions of individuals delegate the reproductive role to one or few queens, while nonreproductive workers carry out all tasks required for colony maintenance (*1*). These mutually exclusive morphologies and behaviors arise from a single genome and are typically dictated not by genetic differences, but by environmental factors (*2*). The first fertilized (diploid) eggs laid by a founder queen develop into workers, but as the colony enlarges, some diploid embryos take a different developmental path to become virgin queens, which leave the nest, mate, and establish new colonies. As colonies mature, queens transition from a broad behavioral repertoire that allows them to forage, excavate nests, and rear offspring, to one restricted to egg-laying and total dependence on workers. Queens also live up to 10 times longer than workers and 500 times longer than males (*3*).

We compared the genomes of the ants *Camponotus floridanus* and *Harpegnathos saltator*, because of contrasts in their behavioral flexibility, caste specialization, and social organization. *C. floridanus* lives in large organized colonies, in which only the queen lays fertilized eggs; when the queen dies, so does the colony (*1*). Nonreproductive individuals belong to two separate castes, major and minor workers, which exhibit differences in morphology and behavior established during development purely on environmental grounds. In contrast, the *H. saltator* social system and division of labor are more basal: dimorphism between queens and workers is limited, and when the queen dies she is replaced by workers that become functional queens, called gamergates (*4*).

These two ant species differ in other respects as well. *C. floridanus* are scavengers, forage diurnally and nocturnally, and lay pheromone trails that mark paths to food sources. *H. saltator* workers prey on small arthropods in a solitary and diurnal fashion. *C. floridanus* exhibits high territoriality, strong nestmate recognition, and elaborate task specialization. In contrast, *H. saltator* displays low territoriality, loses nestmate recognition in the laboratory, and has only basic task specialization.

The Illumina Genome Analyzer platform was used to sequence genomic libraries for *C. floridanus* and *H. saltator*, obtaining more than 100-fold coverage. Draft genomic assemblies reached scaffold N50 size of ~600 kb (table S1), although for *C. floridanus* most genome-wide analyses reported here were conducted on an earlier version (v3), with scaffold N50 size of 444 kb (table S1). Assembly resulted in only small gaps and large N50 size, which assured us that most genomic features, particularly gene models, were predicted with reasonable accuracy. We verified the assemblies by sequencing 9 (*C. floridanus*) and 10 (*H. saltator*) randomly selected fosmid inserts (average size, 37 kb) (table S2). Additionally, we sequenced ~5000 expressed sequence tags from each ant and mapped them to the assembled scaffolds; more than 95% matched the assemblies (table S3).

The *C. floridanus* and *H. saltator* assemblies cover more than 90% of the genomes, which we estimate at 240 and 330 Mb in size, respectively (fig. S1 and table S4). The *H. saltator* assembly contains 45% G+C, similar to *Drosophila melanogaster* (42%), and *Nasonia vitripennis* (42%), whereas the *C. floridanus* genome is A+T rich, with a G+C frequency of 34%, similar to *Apis mellifera* (33%) (*5*) (table S4). Organisms that use DNA methylation for gene regulation typically display a depletion of CpG dinucleotides in their genome (table S5); however, CpG dinucleotides are overrepresented in both ant genomes

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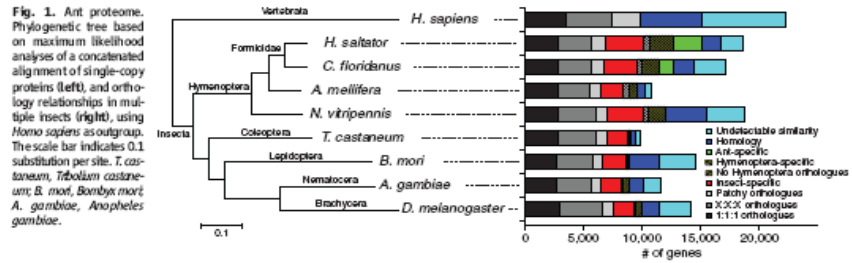

Fig. 1. Ant proteome. Phylogenetic tree based on maximum likelihood analyses of a concatenated alignment of single-copy proteins (**left**), and orthology relationships in multiple insects (**right**), using *Homo sapiens* as outgroup. The scale bar indicates 0.1 substitution per site. *T. castaneum*, *Tribolium castaneum*; *B. mori*, *Bombyx mori*; *A. gambiae*, *Anopheles gambiae*.

points, but was not seen at earlier time points or in the two unexposed control birds. This may be indicative of antigen or viral exposure or an abortive infection.

These data show that the TG-D5 chickens did not efficiently transmit infection to birds housed with them, but the specific mechanism underlying this effect is not known. Polymerase decoys may disrupt replication by direct binding to polymerase or indirectly by influencing the level of expression of the recently discovered, putative regulatory small viral RNA molecules (*14*, *15*) (which may also have a role in innate immunity). Although decoy 5 suppressed polymerase activity in cell culture, this did not translate into a quantitative reduction in virus shedding from infected birds (Fig. 2) (nor have we found any effect in ovo or in fibroblast cell culture). Polymerase-RNA interactions may be involved in the virus packaging process, but after passage through TG-D5 chick embryo fibroblasts in cell culture, we have not found any effect on the genome plaque-forming unit ratio of the virus to support the hypothesis that the decoy induced the formation of defective virus particles. The standard intravenous pathogenicity index of the virus shed from one of the TG-D5 chickens (#4457, dpi = 2) was determined after a single passage in embryonated hens' eggs and found to be unaltered, indicating that passage through TG-D5 chickens does not rapidly select for a stable genetic change that reduces the virulence of the shed virus.

Our goal was a proof-of-principle demonstration that genetic modification can be used to prevent avian influenza infection in chickens. The TG-D5 birds exhibited a marked absence of onward transmission of infection, even to unprotected (nontransgenic) chickens housed in direct contact with them. This property could have a major impact on susceptibility and propagation of infection at the flock level and supports the concept of genetic modification for controlling AIV infection in poultry. Our strategy offers substantial potential benefits over vaccination. Although conventional AIV vaccines can achieve strain-specific clinical resistance to primary challenge, sterile immunity is not achieved (*3*). Such vaccination can allow the cryptic circulation of virus in flocks, facilitating antigenic drift and posing a risk to unvaccinated birds and humans that come into contact with them. In contrast, onward transmission and circulation at the flock level are absent in the TG-D5 chickens. The decoy 5 RNA corresponds to an absolutely conserved sequence that is essential for the regulation of viral transcription, replication, and packaging of all subtypes of influenza A virus, offering pan-subtype A protection, whereas vaccination offers no protection against unmatched viral strains. Unlike proposed micro-RNA-based strategies (*4*, *5*), the development of resistant virus is intrinsically unlikely, requiring mutations in the polymerase and the promoter of all eight genome segments simultaneously, a statistically highly improbable event.

The control of avian influenza by genetic modification brings obvious health benefits to consumers and producers, as well as welfare and productivity benefits to the birds. Nevertheless, it is important to assess any genetic modification for potential hazards. Here, the transgene encodes an innocuous decoy RNA, expressed at steady-state levels that are barely detectable by conventional methods and unlikely to present a risk to consumers, birds, or the wider environment. There are no apparent ill-effects on uninfected transgenic birds, which are phenotypically normal and show no significant deviation from the expected Mendelian frequency or differences in hatch weights (fig. S4 and table S2). The transgene is not expected to alter susceptibility to other pathogens, although this has yet to be confirmed. Transgenes can be introduced into multiple founder lines as discrete traits without affecting other genetic properties of the lines. This will facilitate the permanent introduction of novel disease-resistance traits into the mass population of production birds via conventional breeding techniques, with little impact on genetic diversity or valuable production traits. Our approach is technically applicable to other domestic species that are hosts of influenza A, such as pigs, ducks, quail, and turkeys. Further development of transgenic disease resistance in poultry and other farm animals will undoubtedly stimulate debate about the application of this technology in food production.

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Human Tears Contain a Chemosignal

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Emotional tearing is a poorly understood behavior that is considered uniquely human. In mice, tears serve as a chemosignal. We therefore hypothesized that human tears may similarly serve a chemosignaling function. We found that merely sniffing negative-emotion–related odorless tears obtained from women donors induced reductions in sexual appeal attributed by men to pictures of women's faces. Moreover, after sniffing such tears, men experienced reduced self-rated sexual arousal, reduced physiological measures of arousal, and reduced levels of testosterone. Finally, functional magnetic resonance imaging revealed that sniffing women's tears selectively reduced activity in brain substrates of sexual arousal in men.

Charles Darwin suggested that expressive behaviors initially served emotion-relevant functions, before evolving to serve as emotion-signals alone (*1*, *2*). Thus, the behavior of emotional tearing, considered uniquely human (*3*), is a paradox: Whereas tears clearly serve as an emotional signal (*4*), tears were not related to any emotionally relevant function. Despite psychological theories on the meaning of tears (*5*, *6*) and biological theories describing tears as an adaptation related to their eye-protective nature (*3*) or a mechanism for expelling toxic substances (*7*), the functional significance of emotional tears remains unknown (*8*).

Tears are drops of liquid produced by the lacrimal, accessory lacrimal, and Meibomian glands, which contain proteins, enzymes, lipids, metabo-

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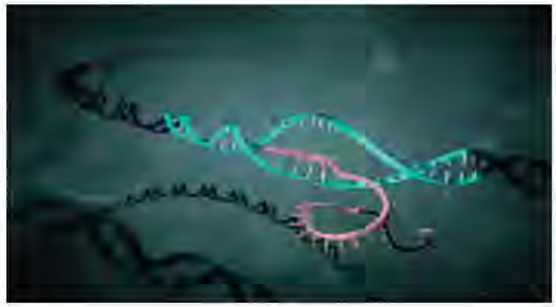

Shining a Light on the Genome's 'Dark Matter'

IT USED TO SEEM SO STRAIGHTFORWARD. DNA told the body how to build proteins. The instructions came in chapters called genes. Strands of DNA's chemical cousin RNA served as molecular messengers, carrying orders to the cells' protein factories and translating them into action. Between the genes lay long stretches of "junk DNA," incoherent, useless, and inert.

That was then. In fact, gene regulation has turned out to be a surprisingly complex process governed by various types of regulatory DNA, which may lie deep in the wilderness of supposed "junk." Far from being humble messengers, RNAs of all shapes and sizes are actually powerful players in how genomes operate. Finally, there's been increasing recognition of the widespread role of chemical alterations called epigenetic factors that can influence the genome across generations without changing the DNA sequence itself.

The scope of this "dark genome" became apparent in 2001, when the human genome was first published. Scientists expected to find as many as 100,000 genes packed into the 3 billion bases of human DNA; they were startled to learn that there were fewer than 35,000. (The current count is 21,000.) Protein-coding regions accounted for just 1.5% of the genome. Could the rest of our DNA really just be junk?

The deciphering of the mouse genome in 2002 showed that there must be more to the story. Mice and people turned out to share not only many genes but also vast stretches of noncoding DNA. To have been "conserved" throughout the 75 million years since the mouse and human lineages diverged, those regions were likely to be crucial to the organisms' survival.

Edward Rubin and Len Pennacchio of the Joint Genome Institute in Walnut Creek, California, and colleagues figured out that some of this conserved DNA helps regulate genes, sometimes from afar, by testing it for function in transgenic mouse embryos. Studies by the group and others suggested that noncoding regions were littered with much more regulatory DNA than expected.

Further evidence that noncoding DNA is vital has come from studies of genetic risk factors for disease. In large-scale searches for single-base differences between diseased and healthy individuals, about 40% of the disease-related differences show up outside of genes.

Genetic dark matter also loomed large when scientists surveyed exactly which DNA was being transcribed, or decoded, into RNA. Scientists thought that most RNA in a cell was messenger RNA generated by protein-coding genes, RNA in ribosomes, or a sprinkling of other RNA elsewhere. But surveys by Thomas Gingeras, now at Cold Spring Harbor Laboratory in New York, and Michael Snyder, now at Stanford University in Palo Alto, California, found a lot more RNA than expected, as did an analysis of mouse RNA by Yoshihide Hayashizaki of the RIKEN Omics Science Center in Japan and colleagues. Other researchers were skeptical, but confirmation soon came from Ewan Birney of the European Bioinformatics Institute and the Encyclopedia of DNA Elements project, which aims to determine the function of every base in the genome. The 2007 pilot results were eye-opening: Chromosomes harbored many previously unsuspected sites where various proteins bound—possible hotbeds of gene regulation or epigenetic effects. Strikingly, about 80% of the cell's DNA showed signs of being transcribed into RNA. What the RNA was doing was unclear.

Other studies revealed that RNA plays a major role in gene regulation and other cellular functions. The story started to unfold in the late 1990s, when plant researchers and nematode biologists learned to use small RNA molecules to shut down genes. Called RNA interference (RNAi), the technique has become a standard way to control gene activity in a variety of species, earning a Nobel Prize in 2006.

To understand RNAi and RNA in general, researchers began isolating and studying RNA molecules just 21 to 30 bases long. It turned out that such "small RNAs" can interfere with messenger RNA, destabilizing it. Four papers in 2002 showed that small RNAs also affect chromatin, the complex of proteins and DNA that makes up chromosomes, in ways that might further control gene activity. In one study, yeast missing certain small RNAs failed to divide properly. Other studies have linked these tiny pieces of RNA to cancer and to development.

The surprises didn't stop at small RNAs. In 2007, a group led by Howard Chang of Stanford and John Rinn, now at Beth Israel Deaconess Medical Center in Boston, pinned down a gene-regulating function by so-called large intervening noncoding RNAs. Rinn and colleagues later determined that the genome contained about 1600 of these lincRNAs. They and other researchers think this type of RNA will prove as important as protein-coding genes in cell function.

Many mysteries about the genome's dark matter are still under investigation. Even so, the overall picture is clear: 10 years ago, genes had the spotlight all to themselves. Now they have to share it with a large, and growing, ensemble.

–ELIZABETH PENNISI

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Vernalization-Mediated Epigenetic Silencing by a Long Intronic Noncoding RNA

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Vernalization is an environmentally-induced epigenetic switch in which winter cold triggers epigenetic silencing of floral repressors and thus provides competence to flower in spring. In *Arabidopsis*, winter cold triggers enrichment of tri-methylated histone H3 Lys^{27} at chromatin of the floral repressor, *FLOWERING LOCUS C* (*FLC*), and results in epigenetically stable repression of *FLC*. This epigenetic change is mediated by an evolutionarily conserved repressive complex, polycomb repressive complex 2 (PRC2). Here, we show that a long intronic noncoding RNA [termed COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR)] is required for the vernalization-mediated epigenetic repression of *FLC*. COLDAIR physically associates with a component of PRC2 and targets PRC2 to *FLC*. Our results show that COLDAIR is required for establishing stable repressive chromatin at *FLC* through its interaction with PRC2.

Developmental fates of cells are determined by innate genetic programs and interactions with their environments. A major environmental cue that plants sense to monitor seasonal change is winter cold, as exemplified by vernalization (*1*). Vernalization is the process by which certain plants acquire competence to flower rapidly in spring by sensing prolonged exposure to winter cold (*1*). In *Arabidopsis*, the stability of the vernalized state results from the stable repression of a potent floral repressor, *FLOWERING LOCUS C* (*FLC*) (*2*). A prolonged exposure to cold induces a plant homeo domain (PHD) finger–containing protein, VERNALIZATION INSENSITIVE 3 (VIN3) (*3*), and VIN3 becomes associated with an evolutionarily conserved repressive complex, polycomb repressive complex 2 (PRC2) (*4*, *5*). PRC2 mediates histone H3 Lys^{27} trimethylation (H3K27me3) through its core component, a histone methyltransferase, Enhancer of Zeste [E(z)] (*2*, *6*). During and after vernalization, PRC2 occupancy at *FLC* increases and correlates with an increased level of H3K27me3 at *FLC* chromatin, which is required for the stable maintenance of *FLC* repression (*2*, *5*, *7*, *8*). In *Arabidopsis*, there are three homologs of E(z). These include CURLY LEAF (CLF), SWINGER (SWN), and MEDEA, which are involved in several developmental programs in *Arabidopsis* (*6*). However, the molecular determinants for the increased recruitment of PRC2 and subsequent establishment of H3K27me3-enriched repressive chromatin at *FLC* by vernalization are not known. We identified a long intronic, noncoding transcript that plays role in the vernalization-mediated epigenetic silencing of *FLC* through the recruitment of PRC2 to *FLC* locus.

Several long noncoding RNAs (ncRNAs) have been shown to target repressive histone-modifying activities and epigenetically silence transcription through a molecular interaction with specific chromatin domains (*9*, *10*). Notably, PRC2-mediated silencing includes the interaction with such ncRNAs (*11*, *12*). A group of related antisense ncRNAs (termed COOLAIR) from *FLC* have been proposed to be involved in vernalization-mediated *FLC* repression (*13*). However, the importance of COOLAIR in the vernalization process has yet to be demonstrated. We independently hypothesized that long ncRNAs may play a role in vernalization-mediated repression of *FLC*, and we identified a candidate ncRNA from *FLC* that is distinct from COOLAIR and that we have designated COLD ASSISTED INTRONIC NONCODING RNA (COLDAIR) (Fig. 1A and fig S1A). We found COLDAIR by using a "tiling" reverse transcription polymerase chain reaction (RT-PCR) approach with more than 100 pairs of oligonucleotide primers to cover the entire *FLC* genomic region during the course of vernalization (Fig. 1A). From this tiling RT-PCR approach, we observed a contiguous batch of RT-PCR products from the first intron of *FLC* that transiently appear during the cold exposure (Fig. 1, A and B).

Unlike COOLAIR, COLDAIR is in the sense direction relative to *FLC* mRNA transcription (Fig. 1A and fig. S1B). COLDAIR contains a 5′ cap structure (fig. S1C), but we did not observe significant levels of COLDAIR in a polyadenylate[poly(A)]-rich fraction of RNA (fig.S1D), suggesting that COLDAIR is not likely to be polyadenylated. Thus, the 3′ ends of COLDAIR were determined by a tiling 5′ rapid amplification of cDNA ends RT-PCR approach (fig S1F). The approximate size of COLDAIR is 1100 bases long (fig. S1, A, F, and G).

The increased level of COLDAIR was observed only during cold exposure with the maximum expression at 20 days of cold, and COLDAIR levels returned to the prevernalized level after more than 30 days of cold (Fig. 1B and fig S2). This transient induction of COLDAIR during cold exposure is similar to that observed for COOLAIR (*13*); however, the induction of COOLAIR peaked at 10 days of cold exposure, earlier than the COLDAIR peak (Fig. 1B).

Deletion of the vernalization response element (VRE) in the first intron of *FLC* impairs vernalization-mediated *FLC* repression without compromising the floral repressor function of *FLC* (*14*). The transcription start site of COLDAIR is within the VRE region (Fig. 1A). To test whether the 5′ region of the VRE (excluding the COLDAIR transcribed region) is sufficient to mediate cold-inducible transcription, we generated transgenic lines in which luciferase is driven by 109 base pairs (bp) of the VRE 5′ region (Fig. 1C). We observed strong luciferase expression after 20 days of cold treatment in multiple transgenic lines (Fig. 1, C and D), confirming the presence of a cryptic promoter in VRE. In addition, luciferase expression remained robust as long as plants were kept in cold (Fig. 1, C and D). Endogenous COLDAIR was induced by ~10 to 20 days of cold (Fig. 1, B and D). However, after 20 days, *FLC* becomes repressed through chromatin changes, and thus COLDAIR promoter regions would become inaccessible to the transcription machinery. Consistent with this interpretation, COLDAIR was expressed well beyond 20 days of cold in *vin3-like 1* (*vil1*)/*vernalization 5* (*vrn5*) and *vernalization 1* (*vrn1*) mutants in which *FLC* repression by vernalization is impaired (fig. S3).

A group of long ncRNAs is transcribed by RNA polymerase V (RNAPV) together with RNA polymerase IV (RNAPIV) to mediate the silencing of constitutively silenced loci in *Arabidopsis* (*15*, *16*). This class of ncRNAs also has a 5′ cap but lacks a 3′ poly(A) tail similar to COLDAIR. However, neither RNAPIV nor RNAPV appears to be involved in the transcription of COLDAIR (fig. S4). Instead, we observed that, although the enrichment of RNA polymerase II (RNAPII) diminished at the *FLC* promoter as plants were exposed to cold, the enrichment of RNAPII at the COLDAIR promoter region transiently increased when expression of COLDAIR peaked at 20 days of cold (Fig. 1E). Thus, it is likely that RNAPII is responsible for the transcription of COLDAIR.

The PRC2 complex, including E(z), interacts with ncRNAs in vitro and in vivo (*11*, *17*). We tested for direct interactions between COLDAIR and the protein components of PRC2 {CURLY LEAF (CLF) and SWINGER (SWN) [E(z) homologs] and VERNALIZATION 2 (VRN2) [Su(z)12 homolog]} recombinant proteins by using in vitro RNA binding assays (Fig. 2, A and B, and fig. S5A). Both E(z) homologs, CLF and SWN, bound to COLDAIR in vitro through the CXC domain (Fig. 2, A and C). However, recombinant CLF protein also bound to antisense COLDAIR (fig. S5B), suggesting non–sequence-specific binding. The non–sequence-specific binding to single-stranded nucleotides (including RNA) by E(z) has been reported previously (*18*). To address the specificity of the interaction between COLDAIR and native CLF protein, we used nuclear extracts prepared from *GFP::CLF*-tagged transgenic lines (where GFP is green fluorescent protein)

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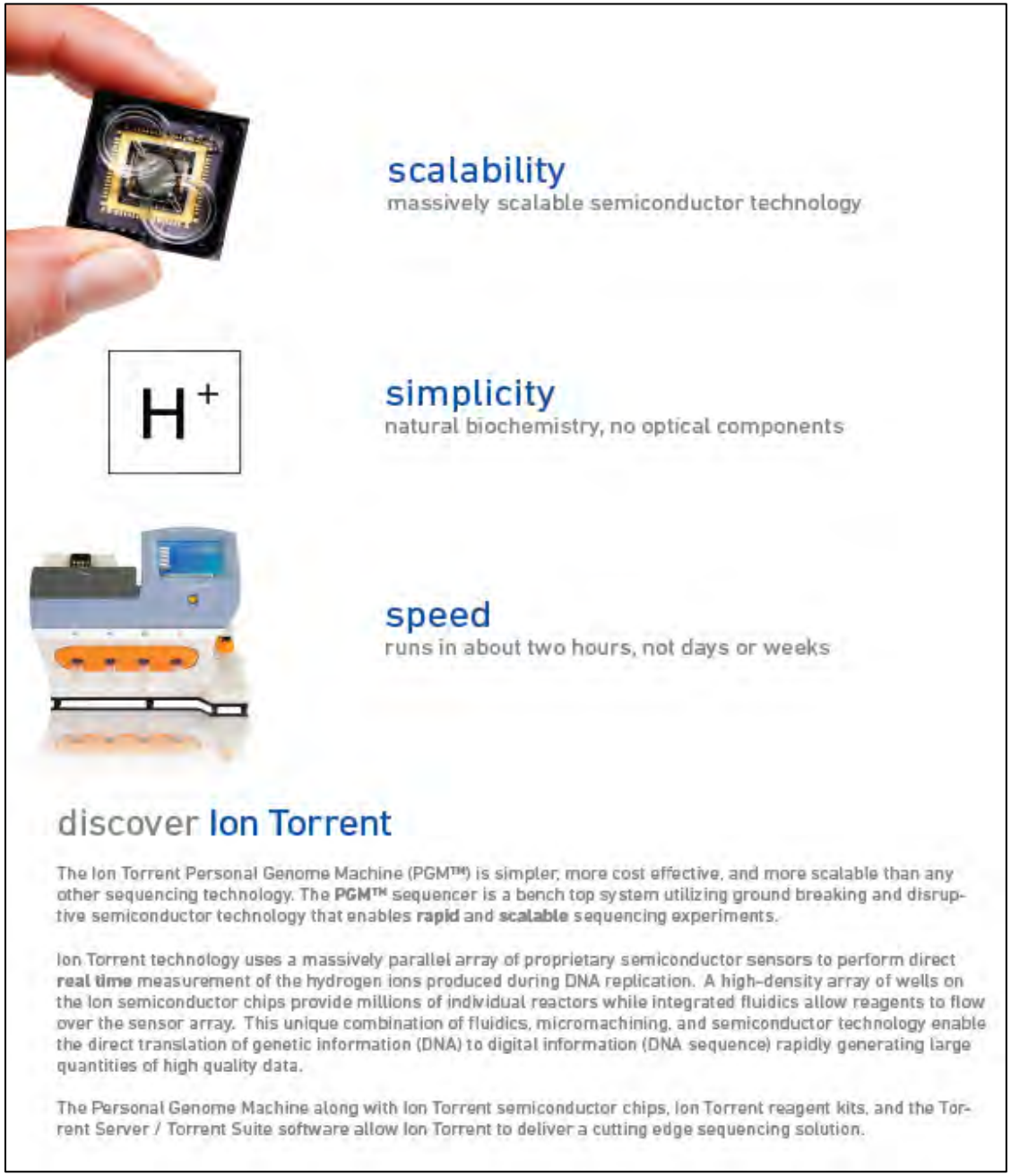

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Ion Torrent seeks "smart" input on new gene machine

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Ion Torrent technology uses a massively parallel array of proprietary semiconductor sensors to perform direct real-time measurement of the hydrogen ions produced during DNA replication. A high-density array of wells on the Ion Semiconductor Chips provides millions of individual reactors while integrated fluidics allow reagents to flow over the sensor array. This unique combination of fluidics, micromachining, and semiconductor technology enables the direct translation of genetic information (DNA) to digital information (DNA sequence), rapidly generating large quantities of high quality data.