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Vanishing honey bees: Is the dying of adult worker bees a consequence of short telomeres and premature aging?

Reinhard Stindl *, Wolfgang Stindl Jr.

Alpharm GesmbH, Sebastian-Kneippgasse 5-7, 2380 Perchtoldsdorf, Austria

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SUMMARY

Einstein is often quoted to have said that without the bee, mankind would have but 4 years to live. It is highly unlikely that he made this comment, which was even mentioned in a Lancet article on honey bees. However, the current vanishing of the bees can have serious consequences for human health, because 35% of the human diet is thought to benefit from pollination. Colony collapse disorder (CCD) in honey bees is characterized by the rapid decline of the adult bee population, leaving the brood and the queen poorly or completely unattended, with no dead bodies in or around the hive. A large study found no evidence that the presence or amount of any individual pesticide or infectious agent occurred more frequently or abundantly in CCD-affected colonies. The growing consensus is that honey bees are suffering from comprised immune systems, which allow various infectious pathogens to invade. The question remains, what causes immunosuppression in many colonies of *Apis mellifera* in North America and Europe? Telomeres are protective DNA structures located at eukaryotic chromosome tips that shorten in the somatic tissues of animals with age. Lifelong tissue regeneration takes place in *Apis mellifera*, and worker bees have been shown to senesce. In humans, a vast amount of literature has accumulated on exhausted telomere reserves causing impaired tissue regeneration and age-associated diseases, specifically cancer and immunosuppression. Therefore, we propose a new causative mechanism for the vanishing of the bees: critically short telomeres in long-lived winter bees. We term this the telomere premature aging syndrome.

Introduction

Albert Einstein is repeatedly quoted to have said: "If the bee disappears from the surface of the earth, man would have no more than four years to live." It is highly unlikely that he made this statement, which was even cited in a Lancet article, titled 'Where have all the bees gone?' [1]. However, the current dying of honey bees has the potential to lead to human health issues, because 35% of the human diet is thought to benefit from pollination [2].

Colony collapse disorder (CCD) was used to describe a syndrome in which many honey bee colonies (*Apis mellifera*) died during the winter and spring of 2006–2007. The phenomenon seems to have spread from North America to Europe and has been reappearing every year since, with varying severity [2,3]. In 2007, some beekeepers experienced 80–100% losses in the United States [4]. The total United States colony loss of 32% over the winter of 2006–2007 increased further to 36% in 2007–2008 and seems to have 'stabilized' at 29% in 2008–2009. The proportion of colonies lost with full-blown CCD symptoms varied between 36% and 60%, over the last 3 years [5]. The characteristics of CCD are the rapid decline of the adult population, leaving the brood poorly or completely unattended, with no dead bodies in or around the hive [6]. There is still plenty of food available and the queen, the brood and immature bees are present. At the final stages of collapse, a queen is attended only by a few newly emerged adult bees [7] with no outward signs of disease, pests, or parasites [6]. Of course, pesticides applied to field crops and to hives have been the first suspects [8]. However, bee poisoning is not very likely in winter and early spring and large numbers of dead and dying bees at the entrance to colonies would be easy to spot [6]. Another blow to the pesticide hypothesis is that the oldest animals in the colony are alive and obviously healthy, the queen. Nevertheless, the first reports appeared that the affected colonies had been left alone by two common parasites: the wax moth and the small hive beetle. The assumption was that CCD might be caused by some toxic residue in the dead colonies. However, as has been suggested by Oldroyd, it seems more plausible that it was simply a consequence of the time of the year (winter and early spring), because the abundance of cleptoparasites is seasonal [6].

In the past, honey bee health was challenged by many parasites, such as varroa mites, honey bee tracheal mites and by fungal, bacterial and viral diseases [8]. Naturally, some groups reported a correlation of certain microbes with CCD. In a metagenomic survey

* Corresponding author. Tel.: +43 680 2150803; fax: +43 1 8697252.
E-mail address: reinhard_stindl@yahoo.de (R. Stindl).

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Cox-Foster et al. found Israeli acute paralysis virus – with a single exception – to be confined to CCD samples [7]. Although this *Science* paper has been cited extensively, the results could not be replicated in a recent expanded study [3].

The growing consensus among researchers is that multiple factors (poor nutrition, pesticides) weaken colonies and make them susceptible to pathogens [9]. Indeed, the observed varying combination of pathogens is not able to explain the losses, suggesting that honey bee colonies are suffering from comprised immune systems that allow pathogens to take advantage of [4,8]. A large study, sampling 91 colonies, found no evidence that the presence or amount of any individual pesticide or infectious agent occurred more frequently or abundantly in CCD-affected colonies [3]. Similarly, the protein content of bees was measured and the results suggested that nutrition does not play a decisive factor [3]. This leads us to the question: what else can cause immunosuppression in *Apis mellifera*?

The hypothesis

The worker population of a western (European) honey bee colony consists of a temporal hive-bee caste that performs a multitude of tasks inside the nest, and a temporal forager caste that specializes in collecting nectar, pollen and water [10]. Depending on the season, the maximum lifespan of worker bees differs significantly (Fig. 1). Whereas the winter worker bees (emerging in autumn) can live for up to 8 months, the worker bees of the remaining year survive for 50 days at the most [10]. Typically, a worker bee feeds the brood for 18–28 days, leaving the hive and collecting nectar and pollen thereafter (foraging). In a natural setting, a forager never returns to the hive-bee stage [10,11]. When all hive bees are removed (for example, by scientists), only a few foragers have been found to revert to caring for larvae [12].

Telomeres are protective DNA structures located at the tips of eukaryotic chromosome (Fig. 2). Each telomere is composed of a repetitive, noncoding DNA sequence and some specialized proteins [13]. Telomeric repeat sequences provide a buffer for the incomplete DNA replication [14] that occurs with each cell division in somatic tissues of animals [15] and in vegetative tissues of plants [16]. In *Apis mellifera*, telomeric TTAGG repeats have been detected on both ends of all chromosomes [17]. Gradual shortening of telomeres is thought to be the source of the limited proliferative lifespan of somatic cells [18]. In humans, a vast amount of literature has accumulated on exhausted telomere reserves causing impaired tissue regeneration and age-associated diseases, specifically cancer, cardiovascular disease and immunosuppression [19–21]. Furthermore, some premature aging syndromes in humans have been linked to shortened telomeres [20,22]. Lifelong tissue regeneration takes place in *Apis mellifera*. In particular, the intestine is characterized by high rates of cell proliferation [23]. It is of special importance to note that worker honey bees have been shown to senesce [24].

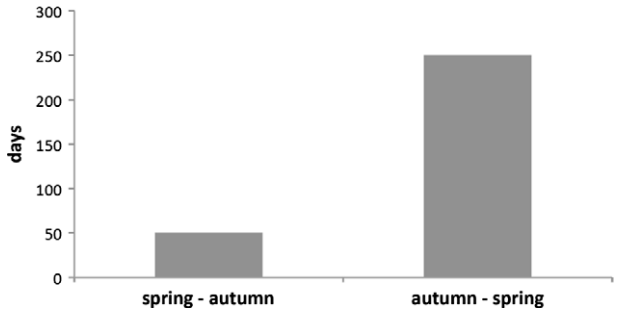

Fig. 1. Maximum lifespan of worker bees depending on the season [10].

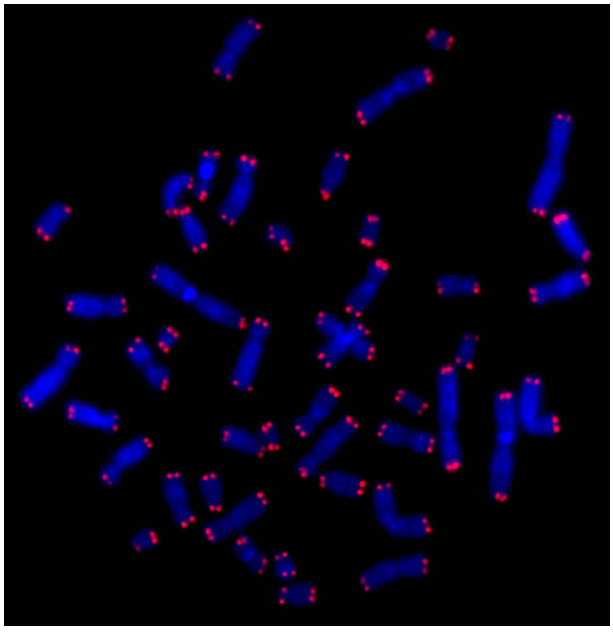

Fig. 2. Telomeres (red) are the protective tips of eukaryotic chromosomes (blue). Cy3-labeled telomeric peptidic nucleic acid probes (*Dako*) hybridized to a normal human metaphase of one of the authors' white blood cells. As can be seen by the varying signal intensity, telomere lengths differ greatly even between homologous chromosomes and have been shown to be a heritable trait [37].

Here, we propose a new causative mechanism for the vanishing of the bees through critically shortened telomeres in long-lived winter bees, and call it the telomere premature aging syndrome (TPAS). Based on the assumption of short telomeres in western honey bees, let us discuss how this could explain some characteristics of colony collapse disorder.

- Can shortened telomeres contribute to increased mortality from infectious diseases? Yes, this has been shown in a human study, published in the Lancet [19].
- If short telomeres lead to immunosuppression and subsequent infectious diseases in old animals, the prevailing sign of CCD should be an unusually young age of the remaining work force. Is this the case? Yes, this has been described as the only characteristic change of the diminished worker honey bee population [3].
- Why does CCD occur in early spring and why does it appear so sudden with no dead bodies in and around the hive? In the movie 'Vanishing of the Bees' (Hive Mentality Films, Los Angeles, 2009) one beekeeper in the United States described the phenomenon in early spring: "The hive was full of bees, three hours later nobody home!" Over the winter season, the lifespan of worker bees increases by 5-fold (Fig. 1), which, according to our proposed model, may lead to advanced telomere erosion caused by regenerative cell divisions in somatic tissues [23]. The winter worker bees might all leave the hive once higher temperatures enable them to fly, because their biological age (shortened telomeres) is too advanced and they cannot resist the impulse to become foragers [10]. They might not return to the colony as a response to an increased pathogen load [25] (suicide hypothesis [26]) or simply die in the field because of their bad health. No worker bees in the hive-bee stage are left, just the few that have emerged newly; finally the colony can succumb to CCD.
- If telomeres become critically shortened in winter worker bees, why is the queen unaffected? Shortened telomeres in short lived males have been found in the ant *Lasius niger*. However,

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in that study the queen and the female worker ants had similar telomere lengths despite a huge (almost 10-fold) difference in lifespan. Obviously, between these two different castes telomere length is not the decisive factor that cause the difference in lifespan [27]. However, in western honey bees all worker bees share the same phenotype throughout the year and are members of the same caste; nevertheless there is a several-fold difference in lifespan between worker bees at different seasons. Thus, in this setting, shortened telomeres might be an issue for life expectancy of winter bees.

- If critically shortened telomeres are present in current colonies of *Apis mellifera*, what made them short? One of us (R.S.) has developed a theory of species extinction and speciation based on ever-shortening telomeres between generations of a species [28].
- Why did the first reports of CCD come from the United States? The queen is the only fertile female in a colony and it mates with several drones from different hives only once, early in life. Spermatozoa are stored in the queen's spermatheca (organ of the reproductive tract) and can be used for several years. Honey bees are not native to the American and Australian continents and the increased colony splitting activity carried out by humans might have led to a slightly higher number of queens' generations compared with their counterparts in Europe within the same time frame.
- Recently, a microarray analysis revealed increased amounts of polyadenylated ribosomal RNA fragments in diseased honey bees [4]. Is this compatible with the telomere model? A markedly increased expression of polyadenylated rRNA transcripts in insects has been found to be inversely associated with age in the termite *Reticulotermes flavipes* [29]. Thus, a high level of these rRNAs in surviving honey bees of affected colonies seems to be simply a consequence of the unusually young age of the remaining work force.
- Why is there a non-random distribution of affected colonies? In other words, why are dead and weak colonies more likely to abut each other [3]? All bee colonies of a beekeeper tend to be related to each other and have originated from one or a few queens. As a consequence, all related colonies in a local area might have to deal with the burden of shortened telomeres.
- Could shortened telomeres be involved causally in other widespread losses of honey bee colonies, in the absence of full-blown CCD symptoms? Yes, any dying of bees with symptoms of increased pathogen load or signs of premature aging should be investigated in regard to telomere length, even if the bees die within the hive. In fact, we expect that shorter telomeres in future generations to kill the winter bees before higher temperatures allow them to leave the colony.
- Will telomere erosion cause extinction of western honey bees? According to the telomere speciation model [28], this is not very likely. Subsequent to the initial phase of chromosomal instability, the occurrence of chromosomal polymorphisms and the creation of a new chromosomal race should allow some colonies to recover and gain fitness over following generations. The queens, holding the new fused chromosome in their eggs, cannot utilize the spermatozoa of drones with the "old" original karyotype from other hives (reproductive isolation). Therefore, there will be some sort of inbreeding leading to telomere elongation, as has been documented in lab mice [30].

Proposed experiments to test the hypothesis

To evaluate the hypothesis, one has to compare telomere lengths of unaffected and affected bee colonies in the same geographical region. All currently available methods to delineate telomere length have limitations and do not measure what is presumably the most important parameter: the few shortened telomeres in a cell that might result in senescence [20]. However, a sufficiently high number of colonies tested might ensure arriving at a statistically significant correlation between short average telomere length (Southern blotting) and the probability of occurrence of CCD. We suggest sampling pupae with a fully formed head capsule with either white or brown eye color [31] to guarantee standardized conditions regarding the ages of individuals between colonies. For Q-FISH experiments [32] bee cell cultures can be established as described by Hunter [31] or primary cells can be prepared from cerebral ganglia of worker prepupae [33].

A more crude approach would be to measure the mean telomere length of different subspecies of *Apis mellifera* and eventually even compare with wasps. This would allow some determination of 'normal' telomere length in this Order of insects.

The diploid chromosome number of *Apis mellifera* is 32 [34,35]. If telomere erosion indeed leads to chromosomal instability in this species, one would expect to see chromosomal polymorphisms in many colonies. The prevailing chromosomal change in evolution is fusion of two acrocentric chromosomes to form a metacentric one. Accordingly, the chromosome number would vary and different chromosomal races might be present, as has been extensively documented in the house mouse [36]. Thus, karyotyping of honey bee colonies at different geographical locations is crucial.

A temporary solution to the problem

A survey of telomere length in all relevant subspecies of *Apis mellifera* has to be undertaken and the strain with the longest telomeres should be chosen for breeding. However, it is the authors' opinion that after a few years of crisis some colonies will gain fitness on their own. Most likely, the diploid chromosome number of these strains will be reduced from chromosome fusion events.

Conflicts of interest statement

None declared.

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