1. Biology and Life Cycle of *Varroa destructor* and Interaction with *Apis mellifera*
    a) Shifted from *Apis cerena* (eastern honey bee in S and SE Asia) to *Apis mellifera* (European honey bee) during the early 1900s likely as European bees were brought to eastern Russia and picked up the mite.
        - Because it is relatively new, most strains of bees are not resistant to them.
        - It has spread worldwide, even Australia, the last varroa-free zone.
    b) Physiology
        - Mites cannot live alone, only in tight relationship with bees.
        - Female mites are wider than long with adapted legs to attach to the bee.
            - They are reddish brown and the only ones you're likely to see.
            - Females have a genital opening—located between the second pair of legs—to accept sperm and release eggs.
            - Females are covered in hairs to sense their surroundings.
        - Male mites are pear shaped and smaller than females.
            - It transfers sperm to the female via one of its digits.
            - Males only live within the brood cell and die soon after mating.
    c) Lifecycle
        - **1.** Female mites are brought to the hive by riding under the sternites (abdominal plates) of a drifting or robbing bee.
            - While attached, they drink substantial amounts of haemolymph (bee blood).
            - Mites know to leave a heavily infested hive: when heavily infested, field and nurse bees put out a similar chemical profile and the female mites then hitch a ride away from the infested colony (Cervo et al. 2014)
        - Using their sensory organs, they seek out nurse bees for transport to brood cells (this population is most-infected bees).
        - Varroa prefer drone larvae because they have more time inside.
            - Mite brood cycle is 12 days in worker cell or 14 days in drone cell.
        - **2.** Female Varroa enters brood cell just before capping (5^{th} instar stage), goes to the bottom of the cell (perhaps to avoid detection), larva finishes its food and then the mite eats haemolymph of larva, activates ovaries, and produces an egg every 30 hrs, up to five in a worker cell and six in a drone
            - First it produces a single haploid male; goes from egg to adult in 5.8 days.
            - Next, diploid full-sister females laid and mature in 6.6 days.
            - Mother mite scratches the bee larva on its fifth segment until it bleeds, upon which the mite larvae can feed.
            - The male matures first and waits in the "fecal accumulation zone" at the bottom of the cell.
            - About 20 hr later, the first female matures and goes to visit its brother for mating.
            - As the next female matures, the process is repeated, often multiple times, until up to 35 spermatozoa are transferred.
            - If more than one female enters a single brood cell, their offspring may intermix. This only happens as mite population grows larger than the brood population.
            - It is possible for a single mite to reproduce itself ad infinitum – extreme inbreeding.
        - **3.** As the larva matures and emerges, the male varroa dies; now-mated female offspring leave the cell and enter a new one to repeat the cycle.

        - A female may complete this cycle up to 7 times in the lab and 2 or 3 times in the wild.
        - Reproduction rate is surprisingly low 1.3–1.45 in worker brood and 2.2–2.6 in drone.
    - Mites prefer temperatures between 26 and 33°C/79–91°F, and some suggest varroa mites seek out peripheral brood because it is cooler.
    - Feeds on the haemolymph (blood equivalent) of bee brood and adults.

d) Interaction with Honey Bee
    - Individual Effects
        - Larvae are parasitized by growing mites eating hemolymph.
        - Bees have lower weight (7 percent for workers, 11–19 percent for drones).
        - Shorter lifespan, less ability to learn, poorer foraging.
    - Disease Vector – Greatest Threat
        - All transmittable by mites: Kashmir Bee Virus, Sacbrood Virus, Acute Bee Paralysis Virus, Israeli Acute Paralysis Virus, Deformed Wing Virus.
        - It seems to be the main vector of Deformed Wing Virus and removal of mites drops an infected colony's instances by 1000 times (although it continues to be transmitted through some other unknown line) (Locke et al. 2017).
        - Like ticks: as they feed on hemolymph, they can share viruses from bee to bee.
        - Also suppresses immune system, which may allow latent viruses to become activated (Shen et al. 2005).
    - Colony Effects
        - Identified correlation between mite infestation and overwintering deaths in German Bee Monitoring Study (2008). Now, were weak hives more easily infected or were infested hives made weaker?
        - Varroa contribute to colony collapse disorder: in addition to Nosema, pesticides, neonicanatoids, climate change, and management practices.
        - Infestation reduces drones and therefore likelihood of successful mating.
        - Infestation reduces swarms and therefore fewer colonies.

2. Treatment
    - If left completely untreated, a colony will succumb in 3–4 years, especially in temperate zones (mite pressure weakens hives in winter; in tropical locations weakening can be overcome by constant growth).
    - Nguyen et al. (2009) conclude that common treatments applied by beekeepers are ineffective in the long term.

b) Identifying Mite Levels
    - Usually expressed as a percentage of bees with mites per 100 bees, e.g., 3 percent is three mites per 100 bees.
    - Direct Sampling: Use uncapping fork to open 100–200 random capped brood cells (especially drone cells) and count how many are infested (Apr-Jun, treat at 4 percent; Jun-Jul, treat at 3–7 percent; Aug, treat at 5–10 percent).
        - Time consuming.
    - Field Bee Candling: catch returning bees and hold them up to the light to see mites attached to the abdomen.
        - Time consuming, not really practical.
    - Alcohol Roll: 1" of rubbing alcohol in bottom of mason jar, 300 bees/1/2 cup bees from middle of brood nest into jar (do not collect the queen); lid on, shake, let bees die; pour

alcohol through strainer onto cloth/paper towel; count mites (6-9 mites means 2-3 percent and suggests treatment).

- Powdered Sugar Roll: collect 300 bees/1/2 cup bees from center of brood nest into mason jar (not he queen); put on lid made of 1/8" screen, add heaping tablespoon of powdered sugar through screen and roll the jar back and forth to coat all bees; wait few minutes and repeat; upend jar and pour out sugar with mites onto tight strainer onto cloth; release bees (6-9 mites means 2-3 percent and suggests treatment).
- Trays under screened bottoms: White board with vaseline, cooking spray, or shelf-liner (sticky side up) placed under 1/8" screen; mites drop off bees onto sticky board; after 24 hr the board is pulled and mites are counted (ca. per day: winter/spring=0.5 mites; May=6 mites, June=10 mites, July=16 mites, Aug=33 mites, Sept=20 mites suggests treatment needed).
- Other signs: shotgun brood, visible deformed wing virus presence.
- Times to measure mite levels: as colony population increases, before and after treatment, and going into winter.
- Threshold for Infestation?
  - Low-level infestations reduce colony population and productivity but do not pose clinical danger.
  - Hard to nail down point at which infestation reaches dangerous level: different for each colony.
  - Generally thought that if the mite population booms as the temperate bees gear up for winter: scattered brood, crippled bees, reduction of population, surprise supercedure.
  - Not a fixed number of mites per colony (bee and brood population variables difficult to account for).
    - Germany study suggests 7 percent infestation (Leibig 2001).
    - US study suggests 3000–4000 mites per colony is dangerous (Delaplane and Hood 1999).
    - Another study says 30 percent infestation during summer results in certain winter death (Fries et al. 2003, Rosenkranz et al. 2006).

c) Hard Chemicals

- Organophosphate coumaphos (Checkmite, Asuntol, Perizin)
  - Interferes with nerve signaling and function.
  - Resistance has been identified.
- Pyrethroids tau-fluvalinate (Apistan, Klartan, Mavrik)
  - Shuts down mite cell function (voltage-gated sodium channels)
  - Popular: easy to apply, effective, inexpensive, but quick resistance can be developed.
  - Resistance recognized two decades ago: desensitized mites; cross resistance to other similarly functioning acaricides (Flumethrin, acrinathrin).
  - Plastic strips applied in bee ways.
- Flumethrin (Bayvarol)
- Formamidine amitraz (Taktic, Mitac)
  - Resistance has been recognized.
  - Will kill bees and has ovicidal effect.
  - 1 ml of 12.5 percent to 10 L water, 80–250 ml/hive, sprayed on bees, combs, hive walls; or fumigated by hanging filter paper soaked in it on a frame, lighting it on fire to smolder, and sealing up the hive for a half hour at night.

- New forms of similarly functioning chemicals is unlikely because similar compounds would meet now-present resistance.
- Things to consider: application itself does not directly jeopardize the honey, but do accumulate in the wax.
    - If bees exposed to multiple compounds stored in wax, they can be harmed.
    - Honey stored in wax can absorb some residues.
    - Residues can remain in wax even after processing.
    - Residue in wax can contribute to later mite tolerance as they breed in wax cells.
    - One study suggests using these chemicals does not seem to cause greater susceptibility of bees to other insecticides they may encounter (Rinkevich et al. 2017).
- Explain Resistance

d) Soft Chemicals
- Formic Acid
    - Placed in hive and allowed to evaporate for two or three weeks, killing mites emerging from brood: small container placed on top of combs in an empty upper with wick filled with 200 ml of 85 percent formic acid for 14 days (longer wick means more evaporation and stronger treatment). Outdoor temp should be 54–77°F, with wide open entrance.
- Oxalic Acid
    - Operates through contact. 35 mg of crystals in 1 L (1:1) sugar water (wear protective gear: glasses, gloves, respirator), syringed in between combs. Should not be repeated.
- Lactic Acid
    - Less bothersome to bees. 8 ml of 15 percent acid sprayed on every side of comb. Can be repeated once per week.
- Thymol
    - 0.5 mg thymol per bee way in a gauze bag hung between combs and left for a few weeks (gets mites emerging, too).
- Advantages:
    - Recognized efficacy, note only formic acid kills mites in sealed cells (rest work on "exposed" mites.
    - Low risk of residues or accumulation (because they are water soluble or volatile).
    - Unlikely to breed resistance.
- Disadvantages:
    - Lactic and oxalic acid must be applied under broodless conditions.
    - Efficacy depends on different applications synced to different environmental conditions within the hive, which can be challenging for home beekeeper.
    - The gap between "effectively kills mites" to "kills bees" is not wide.
    - Efficacy is more variable than hard chemicals.

e) Behavioral treatments
- Removing Drone Brood
    - Taking out 3-4 capped drone frames early in the season reduces mite population 50–70 percent (Charriere et al. 2003).
- Trapping Comb

    - Confine the queen to target combs, remove the sealed combs, destroy mite with formic acid or heat, return the combs.
    - More effective than removing drone brood but labor intensive and detrimental to bee population of hive.
  - Brood Break or Queenless Period
    - Most effective (studies showed 90–100 percent mite reduction in artificially infested hives through broodless period; Calatayud and Verdú 1994; Giacobino et al. 2016).
    - Often noted as "queen replacement" in the literature.
    - Queen is removed or caged and for four weeks the hive has no new brood being lain. Mites continue entering open cells, but on day 9 and 11 the last workers and drones are capped, thus *all mites wanting to reproduce must enter these last open cells.* The larvae die when seven mites are trying to reproduce in a single cell, entombing them. Mites emerging in the interim find no open brood to enter and sit around, loosing viability. It isn't until day 40 or so until new brood begin to be capped, but by then many mites have died and all have reduced fertility.
  - Techniques with "limited efficacy."
    - small cells (tested but no effect seen), screened bottom boards (open so mites fall out, okay for counting mites, not for prevention), powdered sugar treatments (i.e., whole-hive treatment, okay for counting mites in sample), acoustic waves, electromagnetic fields, "energized" water, activated metal disks.
  - Potential new treatments:
    - Introducing a natural pathogen or parasite that attacks the mites; we all know the danger of this and unintended consequences.
    - Entomo-pathogenic fungi (*Metarhizium, Beauveria,* and/or *Verticillium*) seem to be lethal to varroa mites in laboratory tests.

f) Colony Tolerance
  - Natural and Artificial Selection
    - Bees that tolerate mites (*A. Cerana*) control mite populations by halting worker production, which forces mites into drone brood, infested drone brood entomb themselves and do not uncap, thus trapping up to 25 percent of mites. Note that this is unique to Asian bees and the behavior does not seem to transfer to Europeans.
    - *A. mellifera* have developed tolerance in tropical areas (less pronounced seasonality a benefit already) especially with Africanized bees; but when queens moved to temperate conditions they failed to impart tolerance, i.e., it can't be imported (Correa-Marques et al., 2002).
    - A few examples exist of long-term varroa resistance in France (Le Conte et al., 2007) and the US (Seeley, 2007), including the importation of Russian bees thought to have increased mite resistance because of their earlier exposure (De Guzman et al. 2007; Rinderer et al., 2000, 2001, 2003).
      - 10-year breeding program has shown some resistance but it is hard to quantify and control results; also resulted in lower honey production.
      - Another 10-yr artificial insemination program in the US has isolated 10 characteristics likely to reduce mite levels using hygienic bees and other traits for suppressed mite reproduction.
      - In Germany, nontreated colonies are left to produce drones to spread resistant genes to the local population; results not yet in (Büchler et al. 2008; Krause et al., 2007)

    - General pattern of resistance noted that high die-off when mites initially seen and then recovery (Villa et al., 2008).
    - Feral colonies (i.e., swarms that have overwintered without treatment) are good candidates for having some mite resistance).
    - Chemical treatments invalidate natural (or artificial) selection pressures.
- Other Factors
    - Timing and Population Factors
        - Beaurepaire et al. (2017) studied the population of mites vs. bee brood in theoretical and real hives. Because mites prefer a "private cell" they preferentially inbreed. This results in lineages of mite genes within a colony for most of the summer. In the fall, the high mite population is forced to interbreed by sharing cells and worker drift.
            - Take away: if you treat for mites in late summer (before mites outnumber brood cells), you'll select for resistant mites, but if you treat at height of mite population, once they are interbreeding, you'll wipe out more of that resistant gene.
        - Dutch study (van Dooremalen et al. 2012) suggests earlier treatment (July) had a slightly better winter survival than later (August or September) or not at all (although wasn't huge difference).
    - The environment is an indirect influence on mites as they are dependent on bees, which are dependent on the local environment.
        - One study suggests environment is a bigger determiner than management practices: multivariate study of 361 colonies from five ecoregions in Argentina said semi-arid and temperate regions had higher infestations (Giacobino et al. 2017).
    - Grooming behavior (mites removed and killed by bees as they ride on the adults) is practiced by *A. cerana* and has been observed in *A. mellifera* but studies' methodologies (e.g., how this behavior is quantified) and other conditions make it hard to say if this is a trait that can be bred into bees.
    - Hygienic behavior (e.g., uncapping and removing parasitized brood) has mixed results: removal of host larvae doesn't necessarily kill the mites as some escape, it just disrupts reproduction; both Asian and Europeans remove many mites from open brood cells (the latter less so than the former). Unclear if removal of infected brood is because of presence of mites or if it is just because of unfit brood. Unclear if it is environmentally induced or if this trait is heritable.
    - Population factors are also at play: amount of brood and its seasonality, swarming, and brood-free periods need more analysis.
    - Some potential avenues to mite birth control: some brood is less attractive to mites (Africanized and Asian), mite fertility can be decreased for some yet-poorly understood environmental reasons, less drone brood reduces reproduction, shorter post-capping phases reduces reproduction (but allows for another cycle of brood), smaller cell sizes may influence reproduction.
    - New genetic sequencing may soon give us more information about what tolerant bees share with one another and help us promote those traits.
- Major Host Factors that determine mite success include brood availability, drone brood amount, swarming, defensive behavior (and others).
    - Many host factors are influenced by climate and nectar flow.

- Crowded colonies (i.e., multiple colonies within 1 km of one another) increase transfer and size of mite populations.
- Tropical colonies tend to have less collapse due to mites than those that experience winter conditions: likely because the host population is damaged and cannot be revived during the winter.
- Interestingly, royal jelly repels mites (likely due to octanoic acid).
- Mites prefer shorter cells, not artificially long ones; they prefer older, darker cells (probably because they smell stronger); and goldilocks cell size: not to big (e.g., worker in drone cell) or too small (e.g., Africanized cells, which are smaller).
- Mite reproduction declines with multiple mites in a single cell.
- Infertility: 5–20 percent of mites are infertile in European colonies. Originally Africanized bees in Brazil could keep this down to 50 percent infertile, but that has reduced over time.
    - This suggests that hosts can induce infertility somehow, but mechanism is unknown.
    - Starving mites, or ones that have not reproduced in weeks have 2–3 times more infertility.
    - Having multiple mites in a single cell can reduce fertility as well.

g) Recommendations
- If I were dictator of all beekeeping?
    - "The selective breeding of Varroa tolerant bees is considered to be the only long-term solution of the Varroa problem." (Rosenkranz et al. 2010, S107)
        - Baltic Sea experiment: 150 infested colonies kept on island of Gotland without treatment for 10 years. High die-off for first 3 years. Surviving population has recognized low mite levels compared to mainland control colonies. Resulted in lower brood production, more brood diseases (which helps kill off mites during reproduction), smaller winter clusters (thought to reduce amount of mites available in the spring), and less gentle behavior (Fries and Bommarco, 2007; Fries et al., 2003, 2006; Rosenkranz and Fries, 2005; Weller, 2008).
            - Take away: if we select for mite resistance alone, we are basically opening up other things we usually select for: production, gentleness, etc.
    - It has been done in isolated locations but it is complicated because of the multifaceted nature of what resistance means: often a combination of grooming and pupae hygenic behaviors (Moritz 1994).
- Real-world suggestions: use Integrated Pest Management – Have a strategy, don't wait.
    - Monitor, Reduce, Treat
    - Periodic treatment based on mite population growth (in target colony and neighbors).
    - No chemical treatment during nectar flow.
    - Use soft chemical (i.e., naturally occurring) treatments and biological techniques whenever possible.
        - Giacobino et al. (2015, 2016) found in a sample of nearly 400 hives, those that were treated with chemicals and a brood break were best, while chemical only did not fare as well.
        - Wagmotz and Ellis (2010) also showed high efficacy of brood break with OA.
    - In temperate climates treat prior to production of "winter bees," as only robust bees (not parasitized during development) will make it through the winter.

- Use suitable diagnostic tool to determine optimal treatment time (as well as track efficacy and spot flare ups).
- Vary acaricide (rotate different chemicals in time and location) to reduce resistance (Beaurepaire et al. 2017, 54).
    - Note that this is only a short-term solution and will only prolong the colonies, not create resistant ones.
    - Nonchemical treatments should be partnered with chemical ones.
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