JULIE HEFFERNAN

Self-Portrait as Gatherer, 2017
Oil on canvas, 68 x 66 in.

COURTESY CATHARINE CLARK GALLERY

TODD SFORMO

So Much Depends Upon . . .

A biologist and his research methods

We're on the hunt for *Cucujus*—flat bronze beetle larvae hibernating among layers of rotting tree bark. Walking in the woods behind Fairbanks International Airport, a prime spot for sampling them, I tap poplar trees with a chisel, listening for the telltale sound of hollowness, feeling through the metal for the springiness that comes from bark slightly pulled away from inner wood. We're searching for poplars in a particular state of death—not too dead—still clinging on to moisture between layers. In summer, *Cucujus* are found beneath bark in damp, glossy sheets of decaying material that stains fingers and peels away like thin strips of sunburned skin. In winter, the moist grime freezes into large crystals that can trap (even partially) larval bodies for four to five months at a time. It's mid-November, and so far, relatively warm, with lows of -14°C and highs of 2°C. My lab mate Fran Kohl, who works on hibernating Arctic ground squirrels, and I, along with her dog, Jethro, are showing a University of Alaska Fairbanks photographer how we collect these beetles for our study on overwintering physiology of arctic and subarctic insects. We've brought him to this nearby location on a Friday afternoon for a quick field trip. All he needs are a few photos for the university website. Of course, it's not going as planned: we can't locate *Cucujus*, which is strange, since they're usually pretty easy to find in these woods.

Because it's past the time we're supposed to head back, I'm embarrassed by my bad luck of not finding any and start tapping any old tree, even ones where the gnarled, deep grooves of gray bark are several inches away from the inner core, producing a dull, dry thud rather than the tight, hollow echo of slight separation. Every so often, I use my fingers to peel bark that's dangling like old broken shutters, although I know the gap is too large to retain the moisture necessary for *Cucujus* habitat. I'm desperate, though. While prying away bark, I'm almost hit by a three-foot slab above my head that falls, sticking into the snow like a dagger. When I look up, the inner wood is the color of coffee stain and two parallel curly vines creep underneath like varicose veins. Blond sawdust falls, a small amount floats away on the breeze, and I see what looks like black pepper sprinkled on mashed potatoes near the trunk. I figure it's all fibrous miscellany from mold and fugal decomposition, the tunneling waste left by ants, or debris scattered by ransacking woodpeckers.

After returning to the woods and collecting more samples from several different trees, I called Dr. Derek Sikes, curator of insects at the University of Alaska Museum, to ask if I could drop off specimens for expert identification.

Strewn upon the snow, these tiny pepper dots are actually hundreds of mosquito-like insects, some with their large legs extended, others with legs tucked under. Definitely not *Cucujus*, but a lot, maybe a thousand, of something I can't identify, many still clinging to the exposed inner wood, others hanging in loose vertical lines as if joined by thin thread. None are moving, since it's -13°C. Careful not to disturb the scene, I call the photographer, saying confidently, nonchalantly, that I'll be working on these insects this weekend, so he might as well get a few photos. Of course, he asks what they are . . . so much for my bluff. Collecting a bunch in small vials, I say I'll let him know on Monday what I discover over the weekend. Right then, Fran yells, "*Cucujus*!" We get our photos, and I collect some beetle larvae, too, while Fran rounds up Jethro.

I feel a strange eagerness walking into the lab after an experiment has run all night: lofty anticipation that I will be the first to glimpse something unique mixed with lowly relief that nothing broke down (or that I didn't forget to push the record switch). This morning, the computer screen displays eight separate descending lines of color beginning at 0°C, representing temperature of eight out of sixteen individual insects. I programmed the cooling bath to drop to -60°C, since *Cucujus* can resist freezing and supercool below the freezing point of water to very low temperatures. Otherwise, I would have tested the new insect to about -30°C. Good enough. I wasn't going to run a separate bath for a species I wasn't really studying. The temperature traces for the first eight—the important ones, all *Cucujus*—show that they resisted freezing to an average of -41°C (as high as -28.5°C and as low as -53.1°C). No surprise. When I switch to the mosquito-like specimens, however, their slow linear descent to -60°C shows two separate spikes in temperature for each insect, indicating two distinct freezing events occurred per body, with an average of -33°C for the first freezing event and -46°C for the second. I smile. Not a "Eureka!" smile, more how Asimov described the moment of scientific discovery: "That's funny."

In the study of overwintering physiology, there are only a few reports of insects having two freezing events, and no study has focused on them. Right then and there I realize that I can take the weekend, maybe a week, to figure this out; the specimens are handy, just down the street. I'll be one of the first to describe this overwintering ability, to determine which body part freezes while the other part(s) supercools, to understand simultaneous freeze tolerance and freeze avoidance within a single animal. This double freezing phenomenon is something I can sink my teeth into. Winter, however, doesn't last forever, and the overwintering components of many organisms can be seasonal. The darkling beetle *Upis ceramboidies*, for example, is freeze tolerant, freezing at -10°C in summer and winter, but only after acclimating to winter conditions can the beetle be lowered to -70°C for a day and recover within hours. I worry, too, that this new insect might turn out to be an irruptive species, one in which great numbers can be found in some years but not reliably every year. Plus, I'm nearly done with my PhD and delaying another winter to test a new organism is not something my advisors will appreciate, so the race begins.

After returning to the woods and collecting more samples from several different trees, I called Dr. Derek Sikes, curator of insects at the University of Alaska Museum, to ask if I could drop off specimens for expert identification. A relatively recent hire at the time, Derek has gone on to study arctic insects with a vengeance, even the recolonization of insects on the island Kasatochi in the Aleutians after a volcanic eruption covered the island with ash, essentially wiping out life. Fortunately, Derek had collected and documented insects on this island only two months prior to the eruption (Sobel 2017). He narrowed down the range of insect for me—doing away with my less-than-professional description "mosquito-like"—to fungus gnat in the family *Mycetophilidae*. He cautioned me, however, that identifying the gnat to species would require consulting a specialist in *Mycetophilidae*. "You've got to be kidding," I'm sure I said to myself. Of course, there are specialists in species identification, but I was really there are specialists in species identification, but I was really counting on a fast ID. I was glad, though, to have a common name—fungus gnat—to write in my notebook.

Identification, of course, is neither trivial nor merely about calling organisms by their scientific name; it is a schema that places them within an evolutionary web of relatedness, whereby understanding is increased and lessons learned due to relationships, their descent with modification. Along with most other biologists, I can cite Theodosius Dobzhansky in that "nothing makes sense in biology except in the light of evolution," although this does not mean that I'm well schooled in understanding these relationships, which, of course, begin with proper species identification; like others, I rely on taxonomists, an increasingly underfunded group whose work is also undervalued—a bug's a bug, after all. But identification is essential, underpinning the ability to compare apples to apples. The difficulty and importance of identification was illustrated by a fellow graduate student in our overwintering physiology group who worked on *Pterostichus* spp. ground beetles. These small black beetles look very similar, and different species can easily be collected within the same habitat; in fact, they even smell the same when disturbed in the field—an unmistakable odor that, to me, is akin to fermented Magic Marker filtered through dirt and decaying moss. Eventually, the work on *Pterostichus* had to be abandoned, since it was impossible for us to tell the different species apart in the field. In addition to *Pterostichus*, our group tried working on centipedes before finding a taxonomist to identify them. One person, an expert in Italy about to retire, wrote back a few months after receiving the centipedes to say that we were working with more than one species and that it wasn't possible to identify these in the field. Obviously, we had to abandon our work on the centipedes, too.

For help with identifying the gnats to species, I sent out emails to experts I found online, and a few bites came back in the form of "yeah, sure." One reply stood out and sounded something like this: "Can't talk now . . . am in entomology society meeting . . . would be excited and happy to examine these for you." Maybe I should have questioned his lack of focus by emailing me during a meeting, but Dr. Peter Kerr's enthusiasm was what I was hoping for. Always personable in emails, providing more than loose facts, he wrote to me, "The specimens arrived on my desk today. Nicely collected! They belong to the genus *Exechia*." He went on to say, "This is a large Holarctic genus, with well over two hundred species names. There are about fifty species recorded from North America. Offhand, I don't know the species and it will require some work to get it . . . I don't have all of the literature. It seems like an important discovery, however—congratulations!—and a species name will be important. Let me see what I can do. . . ."

It wasn't until February 2, a few months after I had started work on the gnats, that he was able to determine my haphazard collection of specimens from different trees was a single species, *Exechia nugatoria* (Johannsen 1912). What a relief!

Within a month or so of starting to work on this gnat, a short time in terms of scientific research, I was making progress, going from an unknown insect to physiological insight, and only then started my literature review. A paper by Dr. Keith Miller, "Cold-hardiness strategies of some adult and immature insects overwintering in Interior Alaska" (1982), was first on my list. Miller, whom I've never met in person, happened to be a retired professor from the University of Alaska Fairbanks at the Institute of Arctic Biology and now lived in the Lower 48. He has contributed substantially to overwintering physiology of insects. In this paper alone, he relates cold-hardiness features such as freeze tolerance and avoidance, supercooling,

lower lethal temperatures, survival, and seasonal changes in polyhydric alcohols to no less than six orders, fifteen families, and seventeen species of insects from Interior Alaska. I have used the paper not only as reference but as guide. Rereading the paper, which I knew mentioned dual freezing events, I saw *Mycetophila* spp., and my heart sank. While not exactly the same species by name, the difficulty in identification, as noted above, and scientific name changes over time could easily mean that he had already worked on this insect. Furthermore, to the right of the name, two freezing points, one -33°C and the other . . . I couldn't read any further. Dejected, disillusioned, upset at myself for not rereading this paper earlier when I began working on this "new" insect, I put it down in disgust and walked away.

I remember seeing the dull matte finish of bound books on the bioscience library shelves. I knew, of course, they were arranged in an order, but the random colors of their spines now made me mad. Leaving the library, I rode the shuttle around campus, staring out the window, looking at the construction on campus, which younger students would be taking advantage of, and wondering why I was not a better student. I remember sitting in the cafeteria on lower campus, away from the upper campus science buildings, to have lunch alone and pick up the pieces. I was amazed at how much expectation I had unwittingly put into these gnats in such a short time, in trying to make this discovery, in trying to prove myself. I resolved to improve, to take the time to work in a more orderly fashion, to fully read and know the literature and, most importantly, not allow myself to be caught up in games of being first but focus on science. *I could still write a paper about a novel overwintering strategy*, I reassured myself, but it just didn't seem to be the same.

Back in the lab, with my new goal in mind, I grabbed the paper for a more thorough evaluation. Well, instead of rereading it in its entirety (you can see that I'm already skimping on what I said I'd do), I skipped to the section on the gnats and saw the freezing point. Freezing *point*, only one freezing event! I had not even thoroughly read the section on gnats an hour ago. Miller recorded dual freezing events in some insects, but not this one.

Eventually, maybe a month or more later, I found his phone number and called to discuss my findings. I jokingly said that one of my goals was to work on an insect he hadn't already studied—I'm still trying to do that. I asked him if he recalled these gnats, and he described a scene similar to mine when I first pried the bark back and they fell out, adding that he'd always meant to do more work on them since they're so plentiful. While discussing what I had found, I asked why he didn't find dual freezing events, under the assumption that we were working on the same species. Miller said his objective at the time was to focus on freeze tolerance. After he reached the first freezing event, he rewarmed the bath to see if the gnats survived, which they did, so the experiment concluded. During our conversation, it became clear to me that he would have discovered dual freezing events in this gnat had he been less conscientious, and it was my dumb luck that I included gnats in the bath that also had the beetle. By lowering the bath to -60°C because of *Cucujus*, I had stumbled upon the gnat's second freezing event.

Searching for insects in the January woods at 2 p.m., with sunrise and sunset close in time, you experience a whole day's change in light in a matter of hours. Light streams through frosty trees, reflecting subtle blues, golds, and reds of predominately white and black birch bark. Looking up, the heavy green undersides of spruce boughs appear as veins against the skyward side caked with nearly a half a foot of snow. As cold air stings my face and lungs, frosting earlobes and eyelashes, sleuthing out any minute gaps in clothing, I think it can't get much better than this for a low-temperature biologist. Well . . . except for answering this nagging question: which part of the gnat freezes first? Instead of cold air filling my lungs in the midst of a red sunset, my eyes were inches away from the tiny hibernating gnats inside a plastic container set in ice cubes, in a too-warm building, in a lab with walls the color of yellowing glue, and I wondered whether a direct approach—severing the abdomen from the head and thorax—was too drastic (well, for the gnat, of course) for measurement?

Prior to trying this, I had attached two thermocouples per gnat, one on the abdomen and one on the thorax, to see if I could record a difference in freezing times. Since this insect is only about four millimeters long and weighs less than two drops of water, I cut a tip of tapering plastic pipette tube and slid the gnat to the center, observing it under a microscope and delicately arranging the two thermocouple probes onto the segments. The tricky part involved stuffing both ends of the tube with packing foam so that neither the insect nor the probes moved when placed in a beaker that went into a cooling bath. This worked, but because the insect was so small and the release of heat upon freezing was rapid, traveling quickly along a body that was lower than -30°C, there was just a slight offset in the release of heat indicating that the abdomen froze first.

I fully expected that detached parts would not provide reliable freezing points, thinking body fluids would flow as soon as severed. In fact, even slight nicks and abrasions on the cuticle can cause insects to freeze at higher-than-normal subzero temperatures; however, under the microscope, nothing oozed out. I randomly arranged pairs—head-thorax or abdomen—in cooling bath channels and sent the temperature down to -60°C. In the meantime, as the temperature was dropping at 0.2°C per minute (or one degree every five minutes), I used a calibrated fine resolution scale in another lab (the professor was nice enough to lend me a key for after-hours work) to measure fresh body weight of whole gnats and parts. Placing them in an oven at 60°C, I dried the freshly weighed specimens until they reached constant weight to determine water content with the idea that differential water content in different body parts might be reflected in the separate freezing events.

To my amazement and luck, the freezing events of the severed parts duplicated the whole-insect freezing, with the abdomen freezing on average at -30°C (and only a single freezing event) and the head-thorax freezing on average at -50°C (and only a single freezing event). Body water content also cooperated. The whole-insect water content was approximately fifty-seven percent, while the abdomen was approximately seventy-one percent and the head-thorax was approximately forty-six percent. Over a two-month period, I ran supercooling trials before the weather turned bad, i.e., warmed up. Within that time, I answered many questions with sufficient evidence and honed mean supercooling temperature values and I confidently assessed survival. I could cool individuals down past their first freezing event, rewarm them to 0°C, and place them under high humidity in a refrigerator to monitor survival. Survival was good: seventy percent. Past their second freezing event, survival was not: 0 percent. Now that I was getting repeatable results, and the story, I felt, was drawing to a close, I learned it was actually the time when scholars start asking better questions, a time to question not necessarily the results obtained so far but to reconsider methods and interpretations. "How do you know" began a question asked three times independently: my two advisors, Dr. John "Jack" Duman from the University of Notre Dame and Dr. Brain Barnes of the University of Alaska Fairbanks, in whose lab I was doing the work, and independently by Dr. Rick Lee, from Miami University, Ohio, with whom I was corresponding. "How do you know that time spent after the first freezing event is sufficient for ice to pass through the abdomen to initiate freezing (or inoculate) the thorax-head, after all the bath is decreasing at one degree every five minutes?" Or, "What happens if you held the temperature at -40°C overnight?" These obvious questions were coming at a time when I felt I already had answers and a good story. These were the sorts of questions that peer reviewers of scientific journals would ask, of course. Questions, in other words, which could screw up everything. But that's the price one pays for not asking enough questions, not thinking clearly, and relying on answers that appear to be on the right track. It's as if the story in the making were turning out the way it should, and "should" has a tendency to discourage questions.

By the time I got this question, it was already mid-March and ambient temperature was on the rise—and so was the second freezing event. By March 28, the gnats appeared to be acclimating to spring conditions so that the second freezing event increased to -42°C ± 1.3, though I was grateful there was still a separation between the two events, even a statistically significant difference. If I had only thought of testing this potential time lag sooner . . . One month earlier would have done it, for between February 3 and 9, mean ambient temperature was -39.20C ± 0.1, with extremes of -31 and -43°C, so their abdomens would be frozen for six days in a row halfway between the first freezing event and the second lethal one. I had even walked into the woods during these low temperatures to collect insects but never thought of testing the time difference between the first and second freezing. While collecting the gnats, I was especially careful not to clumsily jostle and damage them, using an artist's paintbrush to gently sweep them from the tree bark into a container. I remember wishing some skiers would come by at this moment to witness a (deranged?) person at -35°C in the middle of

the woods painting the interior of a dead tree. Leaving the insects outside near the lab to "warm up" more naturally after the cold snap, I finally checked them on February 18 and found survivorship to be ninenty-one percent (64/70).

I programmed the bath to hold at approximately -35°C for forty-eight hours. After this, it was scheduled to decrease to -60°C, triggering the second freezing event. These temperatures and duration of time seemed reasonable for ice to propagate from the abdomen to head-thorax; after all, these segments were in intimate contact. I posted signs on the bath and in the lab with my phone number in case there was a problem with the instrument. I talked to the late-night maintenance staff: "Call any time." All eyes were on the bath and recorder. The specimens froze at -33°C for the first freezing event and ten out of sixteen individuals exhibited a second freezing event at -39°C on average only after the bath cooled further after holding at -35°C for forty-eight hours. Although not as dramatic as if I had done this a month earlier under more extreme winter field conditions, I now could say something about a unique overwintering strategy in a single insect, simultaneously freeze tolerating and freeze avoiding, a physiological approach more reminiscent of plants (Sformo et al. 2010).

So much for taking a weekend to solve a problem.

I had heard that graduate students should develop something new, a research objective beyond those predetermined by their committee. I never felt that expectation from my committee, which was extremely helpful, because I probably would have grabbed the first thing to come along just to make sure I could check this box off as soon as possible and not give it another thought. The gnats were different. Finding and choosing this insect to study—even though I was nearly done with school—created an opportunity to sum up what I had learned. Strangely, though, this encounter was not a culmination of my education but a beginning, instilling in me a commitment toward exploration, an understanding that new thinking may depend on embracing stray events as a fresh stock of innovative options. And we're still not done with this insect, with its ability; it's science, after all—where questioning and wondering don't end, where seeing can also be a consequent of that which proceeds. We're still wondering why ice does not inoculate the head and thorax after it has formed rapidly in the abdomen; still wondering how differential dehydration is maintained and to what extent it can dehydrate; still wondering what else is out there and which accidental events may lead to something new. More important than answering these questions, the gnat instilled in me a habit of mind to attend to little moments of surprise and has made me in some ways more conscious; it's become a gadfly.

In my lab notebook (early March), I have a memento from this time. While thawing gnats to test survival, one flew the coop when I opened the lid, flying down the hall. Hours later, it was swatted like any old mosquito by Fran, who knew, of course, it had to be one of mine since it was still too cold outside for any insect to revive. She taped the gnat down to a scrap of paper and left it on my desk with a note: "Todd, we don't let our [experimental] squirrels run free, now do we??"

Todd Sformo is a biologist living in Utqiaġvik (formerly Barrow), Alaska, and works on a variety of organisms: bacteria, bugs, bowheads. His PhD, MS, and MFA are from the University of Alaska Fairbanks, and he has an MA in art history from the State University of New York at Buffalo. He has published numerous scientific papers and has two prose poems (or flash nonfiction?), "Knots" and "Gray," published in *Hippocampus Magazine* and *Cirque*, respectively.

JULIE HEFFERNAN

Many Heads, 2019
Oil on canvas, 24 x 18 in.

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