

The “Inch-Worm Episode”: Reconstituting the Phenomenon of Kinesin Motility

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Abstract: New Mechanist philosophical models of "phenomenon reconstitution" understand the process to be driven by explanatory considerations. Here I discuss an episode of phenomenon reconstitution that occurred entirely within an experimental program dedicated to characterizing (rather than explaining) the phenomenon of kinesin motility. Rather than being driven by explanatory considerations, as standard mechanist views maintain, I argue that the phenomenon of kinesin motility was reconstituted to enhance researchers' primary experimental tool - the single molecule motility assay.

Introduction

Following (Bogen and Woodward 1988), the New Mechanist philosophy of science tells us that *phenomena* are targets of explanation in science. Traditionally, in this school, philosophical focus has been on the analysis of explanation, leaving phenomena construed as little more than the targets thereof. Familiarly, mechanistic explanation consists in specifying the organized parts and operations (entities and activities) constituting the mechanism responsible for generating a phenomenon of interest (Bechtel and Abrahamsen 2005; Machamer, Darden and Craver 2000). As (Illari and Williamson 2015) put it:

All mechanistic explanations begin with (a) the identification of a phenomenon or some phenomena to be explained, (b) proceed by decomposition into the entities and activities relevant to the phenomenon, and (c) give the organization of entities and activities by which they produce the phenomenon (123).

However, philosophers have recognized that this gloss on the research process is overly simplistic since (Bechtel and Richardson 1993/2010) coined the phrase “phenomenon

reconstitution” in their seminal work on mechanistic research.¹ Mechanists observe that researchers frequently re-understand an initially identified phenomenon as they acquire insight into the mechanism(s) responsible for it. Mechanist philosophical models of how phenomena are reconstituted in science tend to emphasize the importance of explanatory considerations in driving the process. On such models, phenomena are reconstituted as researchers gain insight into the explanatory mechanisms underpinning phenomena of interest (Bechtel and Richardson 1993/2010; Craver 2007), or as researchers recognize that their favored explanans is better suited to explain a phenomenon occurring at a “level of abstraction” higher than was initially assumed (Kronfeldner 2015). This emphasis is perhaps unsurprising given mechanists’ traditional focus on explanation. That said, a number of philosophers have recently considered the ways in which scientists treat phenomena as objects of investigation in their own right (Colaço 2018; 2020; Feest 2011; 2018). Taking cues from this recent work, I analyze a case of phenomenon reconstitution that occurred entirely within an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin movement.

Research on kinesin—a molecular motor that transports cargo around cells by moving unidirectionally along microtubule protofilaments—involves a substantial amount of experimental work dedicated to characterizing the phenomenon of kinesin movement. Unlike with macroscopic objects whose movements are readily observable, molecular motor movement is a phenomenon that takes place at the nanoscale. Characterizing it therefore presents challenges that require sophisticated experimental tools. In what follows, I focus on a particular tool, the *single-molecule motility assay*. Like patch-clamp recordings that made possible the single molecule investigation of ion channels in neuronal membranes, the single-molecule motility

¹ While the term “reconstitution” may have Kantian connotations for some readers, as it is used by these authors (and myself) no such connotations of the term are intended.

assay enabled researchers to study the kinetic activities of single kinesin molecules and was an invaluable tool in the effort to characterize kinesin movement.

That the appropriate characterization of kinesin movement is that it walks “hand-over-hand” along microtubules was a guiding idea for researchers using the single-molecule motility assay.² In fact, the hypothesis was first suggested in 1989 in the very article reporting the development of this experimental tool. Over the following ten years, data from studies using variations on the basic design of the assay were interpreted as supporting hand-over-hand (HoH) walking, generating a limited consensus that, indeed, the correct characterization of the phenomenon of kinesin movement was that it walked HoH.

However, in 2002, a study involving a particularly interesting variation on this assay briefly disrupted this consensus, making a compelling case that kinesin walks in an “inch-worm” fashion rather than HoH. This study was quickly followed by a number of further single-molecule studies that re-established an even more robust HoH consensus. However, this is not a story of HoH advocates having been correct all along. Rather, the phenomenon of HoH walking was importantly “reconstituted” across the 2002 study.

Section I situates the analysis of the Inchworm Episode presented in Sections II-IV in the context of the broader philosophical discussion of phenomena and provides an indication of how I understand “phenomena” and “phenomenon reconstitution” for the purposes of the analysis to follow. In order to let the case speak for itself as much as possible, I forgo further philosophical discussion until the final section. In Section II, therefore, I turn to directly to the science. I

² This idea guided researchers using other methods as well, in particular, those using traditional biochemical techniques to study the hydrolytic cycle of the kinesin molecule. The interactions between the biochemical and single-molecule programs was important in the effort to map the stages of kinesin’s mechanical steps to stages in its hydrolytic cycle. Further, biochemical work showing that the hydrolytic state of one head limited the hydrolytic activity of the other lent support to the idea that kinesin motility involves “coordinated head activity” Here, I focus principally on the single-molecule program’s attempts to characterize the molecule’s mechanical steps.

discuss the initial battery of single-molecule studies that were taken to support the HoH characterization of kinesin motility paying particular attention to the empirical criteria—*processivity* and *coordinated head activity*—that individuated the HoH characterization as such and informed researchers’ interpretations of their experimental results. Further, I describe the limitations this way of characterizing the phenomenon placed on the probative value of the single-molecule assay. A number of models of kinesin motility could be *conceptually* distinguished that were consistent with the HoH characterization and consistent with extant single-molecule data. However, left without adequate *empirical criteria* to distinguish between these models experimentally, researchers had to rely on indirect, theoretical argumentation to adjudicate between these merely conceptually distinct HoH models. Section III discusses an important 2002 study which exploited the latent experimental significance of ideas forwarded in the context of theoretical debate. This study re-drew the lines along which motility models were individuated, making *torque generation* the primary empirical criterion for individuating models of kinesin motility. This new taxonomy enabled these researchers to design a more probative single-molecule study which led them to reject HoH and forward an “inch-worm” model. Section IV discusses the post-2002 studies that further exploited the new criterion for individuating motility models and secured consensus that kinesin walks hand-over-hand—now reconstituted as asymmetric HoH. Section V relates the terms of my analysis to those of (Feest 2011)’s account of how phenomena are “stabilized” and closes with a discussion of the case in light of extant philosophical models of phenomenon reconstitution.

As will be seen—and contrary to extant philosophical models—the reconstitution of kinesin motility did not occur in the context of attempting to *explain* the phenomenon, mechanistically or otherwise. Rather, it occurred entirely within the context of experimental

efforts to characterize the phenomenon. More specifically, the reconstitution was driven by a recognition that individuating models of kinesin motility in terms of torque generation enhanced the probative value of the experimental program's primary investigative tool—the single-molecule motility assay. With this new taxonomy of motility models in hand, single-molecule researchers were able to use their assay to greater effect and establish a consensus that, indeed, kinesin walks hand-over-hand—now reconstituted as asymmetric hand-over-hand.

Section I: Phenomena in Science

What are *phenomena* in science?³ This is a vexing question addressed differently across sub-circles within the philosophy of science. Discussion of phenomena intersects, in some circles, with traditional issues of concern to philosophers of science (e.g. realism vs. anti-realism and the aim of scientific theorizing). For instance, following Pierre Duhem, constructive empiricists take the aim of scientific theorizing to be to “save the phenomena” where by “phenomena” they mean, as (Massimi 2008) puts it, “empirical manifestations of what there is” (Duhem 1908/1969; van Fraassen 1980). For philosophers of this ilk, the aim of scientific theory is to systematize phenomena under an empirically adequate (as opposed to true) theory—an aim which, it is argued, could be achieved without endorsing the reality of whatever unobservable entities the theory hypothesizes. In contrast to the postulated entities of theory, phenomena are the observable entities, processes, and events the reality of which are taken as given and which are the targets of scientific explanation. Others, (including Massimi 2008), do not attribute to

³ I do not intend to develop a full answer to this question here. My analysis proceeds largely in terms of the New Mechanist view which takes phenomena to be targets of explanation in science.

phenomena the same “given” status and argue that phenomena are “constituted” in a Kantian sense of that term.⁴

Philosophers following Bogen and Woodward (1988) likewise understand phenomena as targets of explanation in science but maintain that many (if not most) of the phenomena of interest to scientists are unobservable. For instance, “the melting point of lead,” “neutral currents” in particle physics, or the “chunking-effect” in human memory research are phenomena which scientists seek to explain but which cannot be observed directly. This view draws support from the fact that a large aspect of the scientific enterprise involves the development of experimental tools and protocols which enable scientists to investigate such phenomena in spite of their unobservability. What are observable, on Bogen and Woodward’s view, are data—the images, readings and values that show up on instrumentation displays and are recorded on data-sheets—which scientists use to draw inferences to the existence and character of unobservable phenomena.

The “New Mechanists” picked up this view of phenomena but moved on quickly to how phenomena are explained—specifically, advancing a mechanistic alternative to the then dominant “covering law” model of scientific explanation. In this paper, however, I am focusing on research devoted to characterizing phenomena, distinguishing it from attempts to explain them. Nonetheless, on the mechanist view—which I take as my starting point—there is a complex relation between phenomena and their explanatory mechanisms. From the point of view of one phenomenon, the organized activity of the components of the mechanism serve as explanation. But the activity of these components can themselves be phenomena. Mechanists

⁴ A sense at least *prima facie* unrelated to that in which phenomena are “reconstituted” according to the mechanists.

make this point in the context of presenting multiple levels of mechanistic explanation (Figure 1).

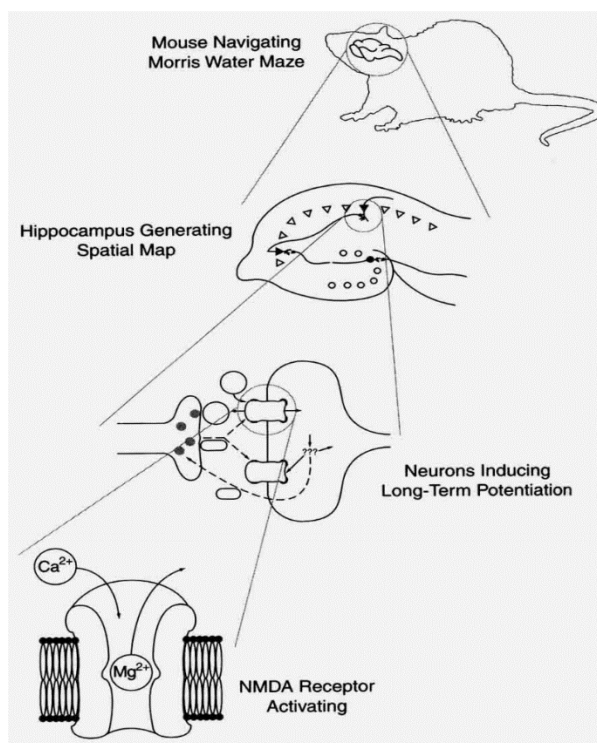


Figure 1: (Craver 2002)

According to the mechanists, in order to explain e.g. the behavior of mice navigating the Morris Water Maze mechanistically, we look down a level at the generation of spatial maps in the hippocampus. In order to explain the generation of spatial maps, we go further down and investigate long-term potentiation (LTP) at the neuronal level. In turn, to explain LTP, we go down to the single-molecule level to understand NMDA receptor activation. As we move down levels, we observe a shift in what is construed as the mechanism and what is construed as the phenomenon. LTP at the neuronal level, for instance, is the mechanism for the phenomenon of hippocampal spatial map generation while, from the point of view of the NMDA molecule, LTP

is the phenomenon to be explained mechanistically at the single-molecule level.⁵ This shifting is part and parcel of the iterative process by which mechanistic explanations are produced on this view. The idea that mechanistic explanation proceeds like this—in terms of levels—is a characteristic feature of New Mechanism, distinguishing it from Ruthless Reductionism which insists that phenomena like mouse behavior in a water maze are explained directly—“in a single bound”—at the lowest molecular level (Bickle 2003).

As expressed in the diagram, LTP is the mechanism from the point of view of one level and the phenomenon from the point of view of another. The phenomenon to be discussed here—kinesin motility—is likewise. From the point of view of explaining the phenomenon of fast axonal transport, the “walking,” cargo-carrying kinesin molecule could be construed as the explanatory mechanism. A specification of the molecule’s *parts* and an account of how they *operate* in an organized fashion so as to bind a cargo and “walk” along a microtubule would constitute a mechanistic explanation for the phenomenon of axonal transport. However, biologists are also interested in explaining mechanistically how the molecule manages to walk in the way that it does. Prior to being able to do so, however, researchers need a characterization of this phenomenon. Does it walk “hand-over-hand?” Does it walk like an “inchworm?” Once it is determined that the molecule walks in *this* way rather than *that*, researchers can seek to understand the mechanical means by which it manages to walk in the characteristic way they have found it to—they can seek to explain the way that it walks mechanistically. In other words, there is 1) the way that kinesin walks and 2) the mechanism by which it walks that way. The

⁵ (Machamer, Darden and Craver 2002) give a canonical statement of this idea: “Mechanisms occur in nested hierarchies and the descriptions of mechanisms in neurobiology and molecular biology are frequently multilevel. The levels in these hierarchies should be thought of as part-whole hierarchies *with the additional restriction that lower level entities, properties, and activities are components in mechanisms that produce higher level phenomena*” (my emphasis).

research discussed below using the single-molecule motility assay was aimed at characterizing kinesin's stepping pattern—the way that it walks—rather than developing mechanistic accounts of the means by which it walks that way. And it did so without recourse to the electron micrographic and crystallographic work aimed at the latter.⁶

The term “phenomenon reconstitution” has not received a formal definition in the literature and I do not intend to formulate one here (although I will return in Section V to discuss it in more detail). That said, to indicate how I understand it for the purposes of the analysis, I first offer clarification of what phenomena are so as to be able to say how they are reconstituted. Phenomena may be understood as answering to a *what* question—*what* is the target of your explanation?⁷ One answers this question by referring to a phenomenon, for instance, “long-term potentiation” or “kinesin's characteristic stepping pattern.” Once a target has been specified in this manner, we can ask “*by what means* does long-term potentiation occur” or “*by what means* does kinesin step in its characteristic way?” As the mechanists have it, these “means-involving” questions are answered at a “lower level” in terms of a specification of the organized parts and operations of the mechanism that generates the phenomenon—the *what*.⁸

Phenomenon reconstitution may be characterized as an event in which there is a change with respect to the answers that researchers would give to a *what* question. For instance, we may ask “*what* is it that you are trying to explain?” To borrow an example from (Bechtel and Richardson 1993/2010) researchers answer, “the Mendelian trait” where “Mendelian trait” is

⁶ In fact, as we will see, at the key moment in the story single-molecule researchers explicitly eschewed data emerging from research at the “lower” explanatory level.

⁷ I thank an anonymous reviewer for inspiring this way of understanding the matter.

⁸ As it turns out, however, the “lower level” mechanistic explanations for molecular motor motility are not specified in terms of parts and operations (entities and activities). Rather, they are given in terms of *constraints* and *energetics*. In a paper currently under review, a collaborator and I argue that in order to make sense of the explanations coming out of mechanistic research on molecular motors, New Mechanism's standard analytic categories of parts/operations or entities/activities are insufficient. Constraints and energetics need to be brought into the mechanistic fold.

understood in a particular way, specifically such that it is identified with a macroscopically observable phenotypic trait of an organism. Now imagine that at some point later in the history of the research program, we ask researchers the same question and they give the same answer but we discern that what is meant by that answer is different from what was meant before. That is, we now ask “what is it that you are trying to explain?” Again, the researchers respond, “the Mendelian trait” but that is now identified with enzymes that are the products of single genes. In such a case, as (Bechtel and Richardson 1993/2010) say, the phenomenon—the *what*—has been reconstituted. As we will see, something very much like this occurred in the case of the phenomenon of hand-over-hand kinesin motility. In fact, detailed scrutiny of this case enables us to understand phenomenon reconstitution in a more philosophically rigorous way. Now, on to the science.

Section II: “Hand-Over-Hand” *circa* 1989 - 2002

By the 1980s, researchers had identified two molecules that function as motors—transforming energy into motion—myosin and dynein. (Vale et al. 1995) identified a third, kinesin, that was responsible for moving cargo such as organelles around the cell interior.

Once kinesin had been identified and named, researchers turned to characterizing its structure and behavior. (Bloom, Wagner, Pfister et al. 1988) subjected purified kinesin to centrifugation, differentiating two heavy and two light chains. They interpreted their results as showing that “bovine brain kinesin is a highly elongated, microtubule-activated ATPase comprising two subunits each of 124,000 and 64,000 daltons . . . and that the heavy chains are the ATP-binding subunits” (3409). Electron microscope studies revealed globular heads at the N-terminal end of the heavy chains, which Scholey, Heuser, Yang et al. (1989) proposed serve both

to bind to the microtubule and to be the locus of ATP hydrolysis. They further hypothesized that the point of having two heads is that one remains attached to the microtubule while the other detaches and moves (Figure 2).

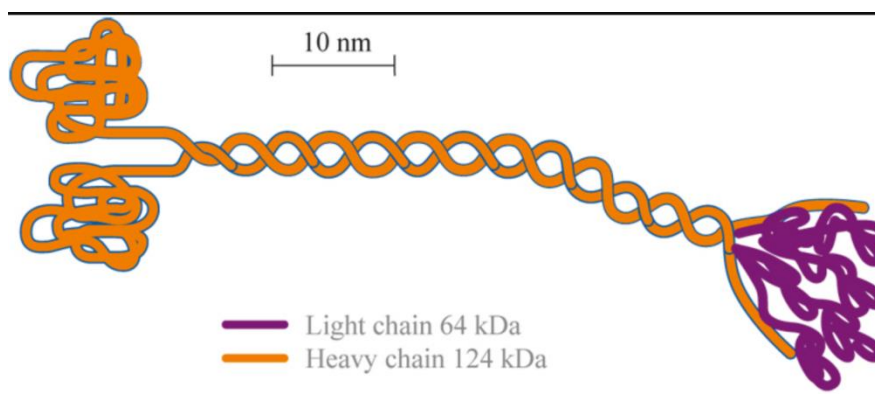


Figure 2: The light chains (right) bind cargo and the heavy chains (“heads” left) bind the molecule to the microtubule and are the site of ATP hydrolysis (“Kinesin Molecule Structure” Shirinsky Vladimir P. https://eng.thesaurus.rusnano.com/wiki/article945?sphrase_id=19463 CC BY 2.0)

Howard, Hudspeth and Vale (1989) (henceforth, HH&V) reiterated this idea suggesting, on the basis of their findings using their newly developed technique for studying individual kinesin molecules, that it walks “hand-over-hand” along a microtubule. As their *single-molecule motility assay* became a central tool for investigating kinesin motility, it is worth explaining in some detail.

In order to develop an assay to investigate the motion produced by a single kinesin molecule, HH&V had first to establish that a single kinesin is capable of moving a microtubule in the first place. Their experimental design inverts how kinesin movement along microtubules may be normally understood—thinking of the microtubule as fixed and the kinesin as moving along it. Inverting this picture, these researchers immobilized kinesin molecules “heads-up” on glass cover slips in solutions containing progressively less kinesin to see how low they could go

and still observe microtubules being moved along the fixed kinesin. Their hypothesis was that if a single kinesin molecule could produce movement, they should observe microtubule movement at very low kinesin concentrations. Initially finding that only when kinesin density exceeded a rather high threshold did microtubules move, these researchers distinguished two hypotheses—first, that kinesin-induced microtubule movement is a highly collaborative affair requiring a number of kinesin molecules working in concert and, second, that kinesin denatures when adsorbed onto the coverslips and only when a sufficient number of molecules are present do a few adsorbed kinesins remain in a conformation that can support movement. Clearly, the first hypothesis, if true, would be damning for the prospects of developing an assay meant to study movement produced by a single molecule.

Optimistically assuming the latter hypothesis, HH&V pre-treated the coverslips to prevent the hypothesized denaturation. Their optimism paid off. They found that they could produce microtubule movement with one-third of the kinesin concentration required with non-treated coverslips. The clincher, however, was the character of the microtubule movement that they observed:

Each moving microtubule rotated erratically about a roughly vertical axis through a fixed point on the surface . . . presumably as a result of thermal forces, or of torques produced when a kinesin molecule bound to different protofilaments. When its trailing end reached this nodal point, the microtubule dissociated from the surface and diffused back into solution (156).⁹

⁹ Notice the mention of “torque.” The idea that HoH walking may produce torque was on the table very early on. As we will see, however, this factor was thoroughly backgrounded in subsequent discussions of experimental results taken to bear on the HoH model of kinesin motility.

The nodal point, these researchers concluded, was a single kinesin molecule. Thus, they found that a single kinesin, immobilized on a glass cover-slip, can move a microtubule and, at the same time, developed a technique for studying this movement that would prove central to the investigation of the phenomenon of kinesin motility. More specifically, they found that a single kinesin can move a microtubule several micrometers. They reasoned that kinesin can remain attached to a microtubule by one of its heads, pushing the microtubule along as the other head moved forward, through 200 – 1000 iterations of its hydrolytic cycle. Linking this finding to the fact that the molecule has two globular heads, these researchers suggested that the molecule works “hand-over-hand” with one head always remaining attached to the microtubule. However, they also suggest an alternative possibility:

It is possible that kinesin’s two globular heads work hand-over-hand, so that *one head is always bound* and prevents the microtubule from diffusing away. Alternatively, the *two heads may work independently* . . . If this is so, the time in the reaction cycle during which the kinesin heads are detached from the microtubule must be so brief, probably less than 1 ms, that the microtubule is unlikely to diffuse out of reach of the kinesin molecule (158 my emphasis).

It's important to attend closely to what “hand-over-hand” meant from the point of view of this 1989 experiment. The contrast HH&V draw between their alternatives makes clear that, as opposed to a characterization on which the heads *work independently* and, thus, on which the whole molecule (both heads) detaches from the microtubule, “hand-over-hand” has it that the kinesin heads *coordinate* their activity and that the molecule remains attached to the MT by at least one head during its walk. In other words, HoH walking consists in 1) the molecule remaining attached to the MT (*processivity*) and 2) *coordinated head activity*. These became the

empirical criteria that were taken by subsequent researchers to individuate the HoH characterization as such and which informed the interpretation of experimental results for the next decade.

Over the course of the following decade, two versions of the single-molecule assay developed. 1) “MT-gliding assays,” like the one already described, in which kinesin molecules are immobilized to glass cover slips and microtubule movement is observed and 2) “bead assays” in which microtubules are immobilized and kinesin-bound beads are observed to move as the kinesin attaches to and walks along the immobilized microtubule. Both “geometries” of the single-molecule assay lent support to both aspects of HH&V’s HoH hypothesis.

Not all studies were immediately univocal in this respect, however. In a version of the bead assay, Block, Goldstein and Schnapp (1990) immobilized microtubules, rather than kinesin, on glass cover-slips. Coating silica beads with carrier protein and exposing them to low concentrations of kinesin, these researchers were able to observe the beads as single kinesin molecules moved them along the immobilized microtubule tracks. Using optical tweezers—which split laser beams to trap kinesins—to individually manipulate the moving beads, they found that under the forces exerted by the optical trap, the bead would detach from the microtubule after, on average, 1.4 μm and be pulled back toward the center of the trap.¹⁰ This, they argued, provides support for the claim that, “the kinesin molecule might detach briefly from

¹⁰ The invention of optical tweezers was significant for research on kinesin motility in ways beyond those discussed here. For instance, since kinesin motility is a phenomenon occurring at the nano-scale, thermal forces are relevant. It is therefore difficult to discern what observed motion is Brownian motion and what is due to the action of the molecule. Having kinesin move cargo against the forces exerted on it by the “trap” ensures that whatever motion is observed is due to the molecule’s action. This technique enabled Svoboda, Schmidt, Schnapp et al. (1993) to observe abrupt transitions of 8 nm steps, a distance that corresponds to the repeat distance between successive α - β tubulin dimers. They propose “that the two heads of a kinesin molecule walk along a single protofilament—or walk side-by-side on two adjacent protofilaments—stepping ~ 8 nm at a time, making one step per hydrolysis (or perhaps fewer, requiring multiple hydrolyses per step).”

the substrate during each mechanochemical cycle” (not processive) and referred to their alternative characterization of kinesin motility as “stroke-release.” (351).¹¹

However, a number of influential single-molecule studies over the next 10 years strongly supported the HoH characterization over the non-processive stroke-release. In a clever variation on the MT-gliding assay, Ray et al. (1993) constructed microtubules consisting of 12, 13 or 14 protofilaments (12-mers, 13-mers, 14-mers). Protofilaments of 13-mers run parallel to the MT axis while 12 and 14-mers exhibit right- and left-handed helical organizations (“twists”) respectively. Observing the movement of these microtubules induced by single immobilized kinesin molecules, the researchers found that the 12 and 14-mers rotated with the pitch and handedness predicted by the hypothesis that the kinesin molecule follows the protofilament axis. That kinesin movement is constrained in this way—that it “tracks the protofilament”—suggested that at least one head remains attached to the MT during its walk, therefore lending support to that aspect of the HoH characterization of kinesin movement.

In a version of the bead assay, Berliner et al. (1995) attached single-headed kinesin derivatives to streptavidin-coated polystyrene beads and found that, unlike intact kinesin or two-headed constructs, the single-headed molecule moved beads perpendicular with respect to the microtubule axis and failed to drive continuous unidirectional movement. This perpendicular movement suggested that the single-headed molecules lack the ability to maintain their association with a particular protofilament track, namely, another head with which to coordinate its activity. The absence of perpendicular movement suggested that the opposite is true for two-headed kinesin, lending support to the idea that the activity of the two heads is coordinated to

¹¹ These researchers also suggested a model on which the molecule is always bound by at least one head but “weakly”—just strongly enough to remain attached in the face of thermal forces, but not strongly enough to remain attached when subjected to the forces of the optical trap.

ensure that one head remains MT-bound at all times. This, in turn assures that the molecule tracks the protofilament axis as it was found to do in the study described above.

Further support for the HoH characterization came with the introduction of fluorescent labelling in the single-molecule assay. In a version of the assay, Vale et al. (1996) directly observed the movement of individual fluorescently labeled kinesin molecules finding that the labeled two-headed kinesin travels an average distance of 600nm per encounter with a microtubule whereas single-headed constructs shows no detectable movement. This corroborated Berliner et al. (1995)'s finding discussed above, suggesting that the two heads working together is required for movement.

Hancock and Howard (1998) immobilized single-headed kinesin onto glass cover slips and found that a minimum of four to six single headed molecules are necessary to produce movement. They further showed that, even at high ATP concentration, the single-headed molecules detached from microtubules 100-fold more slowly than their two-headed counterparts “directly support[ing] a coordinated, hand-over-hand model in which the rapid detachment of one head . . . is contingent on the binding of the second head” (1998). Thus, their study demonstrated a degree of “chemical coordination” between the two heads lending biochemical substance to the idea that kinesin motility involves coordinated head activity.

Single-molecule studies such as these generated a limited consensus that kinesin walks HoH. The empirical criteria that distinguished the HoH characterization (from stroke-release) at this point in the history, are that kinesin walks processively and that it coordinated its heads' activity. The single-molecule assay provided empirical support for HoH insofar as it provided evidence that indeed kinesin is processive and that its heads' activities are coordinated. That said, a number of motility models that met the HoH empirical criteria and were consistent with extant

single-molecule data were *conceptually* distinguished in the literature during this time. However, without empirical criteria by which to distinguish them *experimentally* using the single-molecule assay, it was left to single-molecule researchers to adjudicate between these models by way of indirect argumentation that appealed to data from sources external to the single-molecule program.

To illustrate, (Figure 3) distinguishes five stepping patterns understood to be variably consistent with the data to that time.

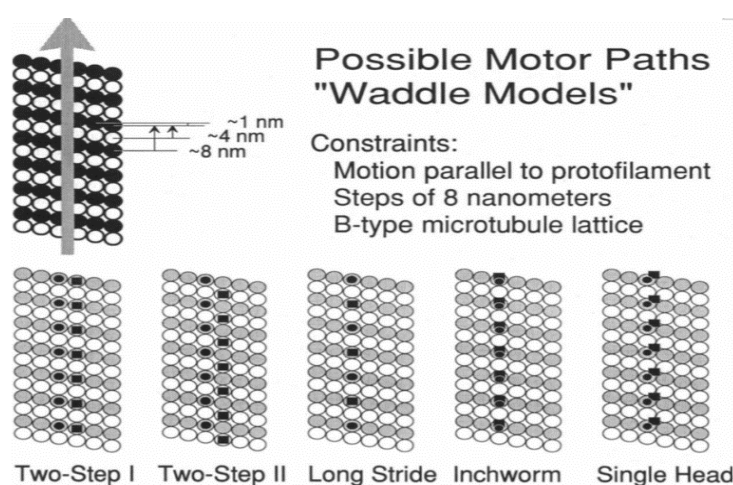


Figure 3: Conceptually distinguished motility models the plausibility of which was left to be adjudicated by indirect arguments based on data coming from outside the single-molecule program (from Block and Svoboda 1995 reprinted with permission)

Findings regarding the structure and dimensions of the molecule, the lattice structure of microtubules and the sites on tubulin heterodimers to which kinesin was understood to bind provided fodder for indirect arguments in favor of or against such conceptually distinguished models. (see Cross, 1995; Howard, 1996; Block, 1998 for reviews).¹² Microtubules consist in

¹²For micrographic data relevant to these indirect arguments see: (Kikkawa et al. 1994; Song et al. 1995; Harrison et al. 1993).

protofilaments arranged in cylindrical fashion.¹³ Each protofilament consists of alternating tubulin (α - and β -tubulin) heterodimers. Several biochemical studies suggested that a tubulin heterodimer can bind only one kinesin head (Song and Mandelow, 1993; Walker, 1995; Tucker and Goldstein 1997). This fact, coming from outside the single-molecule program, was appealed to in adjudicating between conceptually distinct models. For instance, as we see in (Figure 2), an “inchworm model” had been distinguished prior to 2002. On this model, one head always remains in the lead with the other head trailing behind.¹⁴ This model, however, requires each tubulin dimer to have two binding sites (or a single, shared binding site) so that the two heads could be brought into proximity with one another. This, argued Block and Svoboda (1995), was difficult to square with binding patterns gleaned from the aforementioned biochemical studies. They note further that such a model involves an implausibly more complicated step consisting of a “two-part cycle comprising the successive action of both heads” (237). That is, rather than each 8nm step consisting of a single head relocating to the next tubulin binding site, it would involve, first, the lead head moving and, second, the trailing head moving up from behind to keep pace.

These same researchers also argued that “long stride” seemed implausible on the grounds that it required the relatively small kinesin molecule to extend a full 16nm to move the centroid of the molecule 8nm as had been observed in their motility assays. Since this would require that the stalk connecting kinesin’s heads be capable of this kind of extension, Long Stride was deemed speculatively possible at best. Cross (1995) seems to have the same worry in mind in criticizing motility models that require kinesin to stretch its heads across a protofilament,

¹³ Picture the “sheets” in Figure 2 wrapped around to form a cylinder.

¹⁴ Though not a “hand-over-hand” model in what is perhaps the intuitive sense of the phrase, by the lights of the empirical criteria that distinguished HoH models as such (distinguished them from e.g. stroke-release models) “inchworm” models were a species of HoH. As we will see, it was not until the introduction of a new empirical criterion that inchworm models were adequately distinguished from HoH models along empirically tractable lines.

straddling it on either side, and walking along the protofilaments adjacent to it. This would be like “two-step I” only with the squares moved over one protofilament to the right. Cross says of such a model that it is “barely credible” (92).

This kind of indirect argumentation was characteristic of attempts to adjudicate between the motility models that had been conceptually distinguished in the first ten years of single-molecule research. While most researchers agreed that HoH (processivity and coordinated head activity) was the correct characterization of kinesin motility (rather than “stroke-release”), a number of models could be conceptually distinguished, all of which were consistent with HoH by the empirical criteria in terms of which this characterization was specified and all of which were consistent with extant single-molecule data. Thus, a space of merely conceptually distinct models existed to which researchers using the single-molecule motility assay had no experimental access. They were therefore left with indirect argumentation based on findings from sources external to the single-molecule experimental program.

Notably absent from most of this indirect argumentation were considerations of *torque*. This, despite the fact that HH&V had mentioned it in the very paper in which they coined the phrase “hand-over-hand.” There was an exception, however. In an impressively comprehensive review, Howard (1996) did bring the idea that HoH walking produces torque into the discussion along with a number of other considerations the experimental significance of which would be exploited in a 2002 study that represented a significant challenge to the hand-over-hand consensus.

Howard (1996)’s indirect argument represents a compelling theoretical analysis. He assumes, on the basis of analogy with other known molecular motors, that kinesin has a “two-fold axis of rotational symmetry” and infers that, therefore, the heads are functionally equivalent

– “they have the same hydrolysis cycles and make the same motions” (707).¹⁵ He calls this the “equivalence hypothesis.” Tracing out the consequences of this hypothesis in conjunction with extant experimental data, Howard argued that the most plausible model for kinesin motility was a “rotary model” on which the molecule’s heads pass each other on the same side each step (Figure 4) rather than on alternating sides like the way in which our human legs move past each other as we walk.

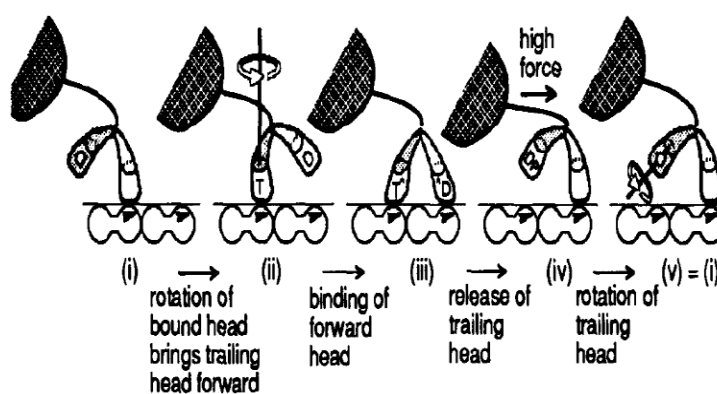


Figure 4: Each head has the same hydrolysis cycle and the same stepping movement with the stepping head always passing the MT-bound head on the same side - notice that state (i) is identical to state (v) (from Howard 1996 reprinted with permission)

His argument involves three key ideas the experimental significance of which was only realized later. First, taking his equivalence hypothesis in conjunction with the protofilament tracking data discussed above, Howard argues against models like the ones labeled **Two-Step** in figure 1. According to such models, the molecule switches back and forth, alternately binding adjacent protofilaments with each head. Assuming the equivalence hypothesis, a consequence of which is that the beginning of each step finds the molecule in the same 3D conformation, Howard argues

¹⁵ For an intuitive sense of what having a “2-fold axis of rotational symmetry” means, imagine two chairs facing each other on either side of a line and equidistant from that line. Rotating one chair 180 degrees with respect to that line will bring that chair into the precise position of its mate. Howard assumed that the relation between kinesin’s two heads was the same.

that if one head (head 1), attached to a protofilament (a) were to undergo a conformational change and motion so as to bring the other head (head 2) to an adjacent protofilament (b), then the equivalent conformational change in head 2 required by the equivalence hypothesis would bring head 1 to the next protofilament over (c). This would induce a rotation in the 13-mer microtubules that was not observed in the single-molecule study discussed above. *Inter alia*, this reasoning leads Howard to his rotary model. As for the second key idea, Howard notes a “seemingly unthinkable” consequence of this model. Because of the assumed equivalence between the heads, the molecule will always rotate in the same direction and “Thus the tail (and organelle) will tend to wind up like the rubber band of a toy airplane” (724). Howard suggests that this torsion could be accommodated by the torsional flexibility the neck was found to exhibit in an earlier study (Hunt and Howard 1993). That the neck has this torsional flexibility is the third key idea.

The experimental significance of these three ideas—1) the equivalence hypothesis, 2) that kinesin motility may produce torque which is communicated to the cargo and 3) that the kinesin neck is torsionally flexible—later came to be appreciated and exploited in a study that introduced a new empirical criterion for individuating motility models. Recall, from the late 1980s to the late 1990s, the empirical criteria that individuated HoH as such were 1) processivity and 2) coordinated head activity. From the point of view of this taxonomy, a number of motility models consistent with the HoH characterization could be conceptually distinguished that were more or less consistent with available experimental data. Adjudicating between them was left a matter of indirect argumentation using data from sources external to the single-molecule program. As we’ll see, (Hua et al. 2002)’s study re-drew the taxonomic lines and, as a result, lent further probative value to the single-molecule motility assay.

Section III: Hand-over-Hand vs. Inchworm

Hua, Chung, and Gelles (2002) inaugurated an important shift in the empirical criteria in terms of which the phenomenon of kinesin motility was investigated. As mentioned above, their study exploited ideas that had been floated in the literature in the context of indirect, theoretical argumentation. First, the design of the experiment was a modified version of (Hunt and Howard 1993)'s assay used to measure the torsional flexibility of the kinesin neck. However, rather than using native kinesin which, in that study, had been found to have a *flexible* neck, Hua and colleagues used a *stiff-necked*, two-headed biotininated kinesin derivative (K448-BIO). This ensured that the connection between the microtubule, this molecule, and the glass cover slip on which the molecule was immobilized would be torsionally stiff, thus guaranteeing that if torque was indeed generated by the walking molecule, as Howard's model predicted, it would not be taken up by a flexible neck. Rather, it would be communicated to the cargo and generate a clearly observable 180-degree rotation of the microtubule with each step of the molecule. Their design, therefore, took the "seemingly unthinkable" consequence Howard had traced out eight years earlier and cleverly turned it into an intervention.

Further, they pointed out that whether the heads of the molecule pass each other on the same side, as in Howard's rotary model, or pass each other on alternating sides, the orientation of the molecule relative to the microtubule axis would switch as the heads alternate between being the leader and being the follower. This, in turn, would generate torque, and induce an observable microtubule rotation. In other words, the differences between the *intermediate* states of rotary models and left-right alternate stepping models were immaterial (Figure 5). What mattered for torque generation was that the molecule *begins* each step in the same 3D conformation only with

the heads swapping between leading and following. Hua et al., dubbed these torque generating models *symmetric hand-over-hand*. By the lights of the criterion of torque generation, both Howard's rotary model and alternate left-right stepping models count as symmetric HoH models.

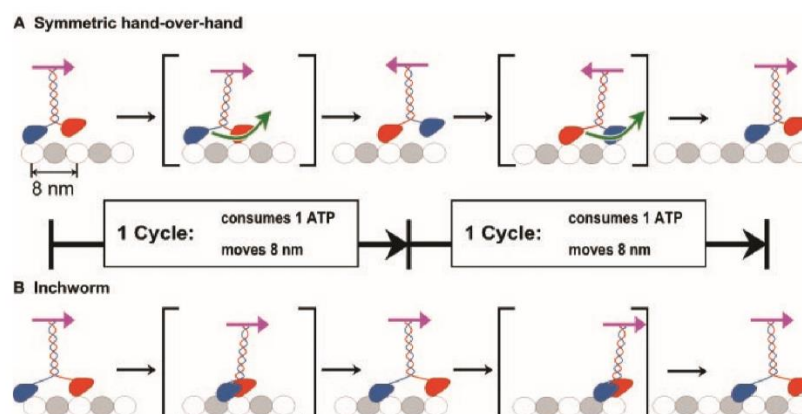


Figure 5: The brackets around the intermediate stages of the steps in A indicate their irrelevance - whether the stepping head passes on the same side, as it does in the diagram, or passes on alternating sides of the bound head, the molecule will change its orientation as indicated by the arrows on top of the molecule (from Hua et al. 2002 reprinted with permission)

To appreciate the shift in criteria for individuating motility models these researchers introduced, consider the sense in which Howard's rotary model would be considered HoH prior to this study. It would count as HoH because it sees the molecule as remaining attached to the microtubule by at least one head (processivity) and that it coordinates the activity of the two heads. The same goes for alternate left-right stepping models. From the point of view of the new criterion—torque generation—both count as HoH but for very different reasons. First off, they would no longer count as HoH full stop. Rather they would be considered *symmetric* HoH to be distinguished from *asymmetric* HoH—a distinction I will discuss in more detail shortly. Further, rather than processivity or coordinated head activity serving to distinguish them as HoH (as opposed to stroke-release), they count as (symmetric) HoH because they generate torque. This, again, for the reason that both view the molecule as beginning each step in the same 3D

conformation, rotating its orientation relative to the microtubule axis during its step and, thus, generating torque.

It was with respect to torque generation that the distinction between symmetric HoH and asymmetric HoH was drawn. Asymmetric HoH denies that the molecule generates torque by denying the equivalence of the heads' steps. For asymmetric HoH, kinesin alternates between *two distinct conformations*—a different one at the beginning of each step—“in precisely such a way as to cancel the 180-degree reorientation induced by head alternation” (847).

Finally, and most importantly, after this re-drawing of the taxonomic lines, “inchworm” was no longer to be considered a merely conceptually distinct HoH model as it was by the lights of the pre-2002 empirical criteria—processivity and coordinated head activity. Now, with torque generation doing the individuating work, inchworm was distinguished from symmetric HoH along empirically tractable lines.¹⁶

Armed with this more probative empirical criterion by which to individuate motility models, Hua et al. (2002) developed and ran their single-molecule assay, failing to observe the microtubule rotations predicted by symmetric HoH models. They therefore rejected that characterization of the phenomenon of kinesin motility. This left two non-torque generating possibilities: 1) that the molecule walks in an asymmetric HoH fashion or 2) that it walks inchworm-style. In a way reminiscent of the indirect arguments discussed above, Hua and colleagues argued against the plausibility of asymmetric HoH. In brief, they found it implausible that the differences between 3D conformations at the start of each step could be such that they

¹⁶ Although it was not empirically distinct from asymmetric HoH as both inchworm and asymmetric HoH were non-torque generating. This is why, as we'll see, these researchers used indirect argumentation to argue in favor of inchworm. As we'll see later, the two became empirically distinct along “limping” lines.

could exactly compensate for the rotation and, in turn, the torque produced by an asymmetric walk.

Interestingly, Hua et al. mention, very much in passing, a cryo-electron microscopy study which investigated kinesin at the “lower” level at which mechanistic explanations for kinesin motility were generated (Hoenger 2000). This study provided some support for the idea that, structurally speaking, the molecule could support the kind of asymmetric walk that Hua et al. found implausible. If considerations at the explanatory level were to have played a role in the phenomenon reconstitution event that I am analyzing, this would be where they would have made their entrance—they would have offered support for asymmetric HoH. But they did not figure into the story. While that study is given a parenthetical reference, Hua et al. ignored its substance. As I said, the “inchworm episode” took place entirely within the context of an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin motility.¹⁷

So, what led these researchers to reject HoH as an appropriate characterization of the phenomenon and adopt inchworm? Note that although their rejection is experimentally motivated, they did not experiment for the purpose of gathering evidence to undermine that which had already been found in support of the HoH model. That is, they did not gather evidence to undermine the single-molecule studies that had supported the claim that the molecule is processive and that its heads coordinate their activity. Thus, they did not employ a “defeater-strategy” as in the case of “memory transfer” discussed by Colaço (2018). Rather, as described above, they recognized the experimental significance latent in certain ideas that had already been floated in the literature. They then constructed a new taxonomy using torque generation as the

¹⁷ Thanks to an anonymous reviewer for pressing me to show how explanatory considerations did not figure into the story.

criterion for individuating motility models which, in turn, enabled them to design a more probative version of the single-molecule motility assay. It further enabled them to recognize an important distinction—that between *symmetric* and *asymmetric* HoH models. Their single-molecule study, they recognized, only bore directly on symmetric HoH models. Their study refuted symmetric HoH, leaving the refutation of the asymmetric model to be done by indirect argumentation. Thus, between their empirical results and indirect argumentation, they rejected symmetric and asymmetric HoH respectively, and defended inchworm as the most plausible characterization for the phenomenon of kinesin motility.

Section IV: Further Experimental Implications of the New Taxonomy

In section II, we noted the role that indirect argumentation played in adjudicating between conceptually distinct models. While such arguments, in addition to the single-molecule data, led to a limited consensus, they were not decisive in adjudicating between the conceptually distinct models consistent with the HoH characterization. However, these more theoretical arguments led to ideas that had latent experimental significance. It was just a matter of unlocking it. The empirical criteria that characterized kinesin motility *circa* 1989-2002—processivity and coordinated head activity—left open an experimental dead-space seemingly inaccessible to the single-molecule assay. The key granting the single-molecule assay experimental access to the dead-space was torque generation. Turning this key generated a new taxonomy and, concomitantly, catalyzed the development of a more probative variation of the single-molecule motility assay.

The studies that emerged in the following two years took advantage of this more experimentally tractable taxonomy, re-securing a consensus that kinesin walks HoH—now

reconstituted as asymmetric HoH. (Kaseda et al. 2003) tested the inchworm model's prediction that only one head is hydrolytically active. These researchers used optical tweezers in a bead assay to measure the stepping rate of kinesins mutated such that one head hydrolyzes ATP more slowly than the other. If both heads are hydrolytically active, they reasoned, their mutant molecule should show a "limp" in its stepping pattern as it walks. This is in fact what they observed undermining the inchworm model's prediction of single-head catalysis. That same year, (Asbury et al. 2003), using optical tweezers in a bead assay, found that kinesin constructs with two identical wild-type heads also show a "limp" in their stepping, suggesting that the molecule alternates between two conformations from step to step. This supported the asymmetric HoH walking model. (Yildez et al. 2004) directly observed the movement of kinesin heads tagged with a fluorescent dye and found that each head moves 16nm per step and also that the tagged heads pause after each movement, presumably while the other untagged head moved. These findings are inconsistent with the inchworm model, which takes each head to move 8nm per ATPase cycle, and supports an asymmetric HoH model. (Higuchi et al. 2004) observed a difference in the timing of every other step in kinesins with identical mutations in the nucleotide-binding sites in each head. The limping they observed is similar to that observed by Asbury and colleagues above, but more pronounced due to the mutation.

Each of these studies exploited the reimagined taxonomy of motility models inaugurated by (Hua et al. 2002). Interestingly, it was no advancement in tool-development that enabled researchers to observe kinesin's "limping" step. The instrumentation necessary to do so—the single-molecule bead assay and optical tweezers—had been in use for over a full decade prior to its being observed. It was rather a conceptual innovation ushered in by the new taxonomy that enabled researchers to look for kinesin's limping step and appreciate its significance. Even if the

limping step had been observed prior, it is not obvious that researchers would have recognized its significance, at least not in the way that it was recognized afterwards. It was in observing kinesin's limp against the backdrop of a taxonomy of motility models which included the category of asymmetric HoH that its significance for experimental work in characterizing the phenomenon of kinesin motility became apparent. Therefore, although recent philosophical efforts to emphasize innovative tool-development in driving experimental research are to be applauded (Bickle 2016), the case of the "inch-worm episode" reminds us that conceptual innovation remains an important factor.

Section V: The "Reconstitution" of Hand-over-Hand Walking

Both before and after 2002, publications in this area of molecular biology regularly refer to kinesin's characteristic stepping pattern as "hand-over-hand." To a casual reader of the literature, it would not be obvious that the phenomenon of HoH walking was reconstituted within the single-molecule experimental program in the way described above. Careful philosophical analysis, however, reveals that what this term meant, as it were, changed across the "inchworm episode" in accordance with the taxonomic shifts that the episode wrought and the concomitant enhancement the single-molecule motility assay's probative value.

Before comparing my account of the inchworm episode with extant accounts of phenomenon reconstitution, let me clarify that when I say the meaning of the term changed, I mean this rather colloquially. In order to spell this out more technically, let me clarify the terms of my analysis and relate them to the terms of (Feest 2011)'s account of how phenomena are "stabilized."

To start, *a phenomenon is constituted under a characterization*. A characterization is specified in terms of *empirical criteria*. *Empirical criteria* individuate the phenomenon along lines experimentally tractable from the point of view of a particular experimental tool. It is just insofar as those models are so individuated that they constitute *characterizations*. So, since a phenomenon is constituted under a characterization and a characterization is specified in terms of empirical criteria, episodes in which the relevant empirical criteria change constitute episodes of *phenomenon reconstitution*.

As we saw in Section 2, the phenomenon of kinesin motility was initially constituted under a characterization specified in terms of the empirical criteria *processivity* and *coordinated head activity*. This occurred concomitantly with HH&V's development of the single-molecule motility assay.¹⁸ It was in the very development of this tool that single-molecule kinesin motility received its initial characterization and, so, was constituted as an object of scientific investigation—a phenomenon. Now, alternative hypotheses regarding the character of the phenomenon could be put forward, tested, supported or refuted.

To clarify further, by “empirical criteria” I also mean those criteria which individuate characterizations (empirically distinct models) with respect to certain supposed features of the phenomenon that are understood or expected to give rise to characteristic patterns of data in the single-molecule assay. Feest (2011) calls such patterns of data “surface phenomena.” As both characterizations and models represent the supposed character of kinesin's movement, they represent what Feest would refer to as the “hidden phenomenon.” For Feest, “stabilizing” phenomena is the process of establishing a “fit” between surface and hidden phenomena.

¹⁸There is a much longer story of how the phenomenon of HoH walking was initially constituted under the empirical criteria of processivity and coordinated head activity. This is the story of how the kinesin molecule was identified in the first place and the single-molecule assay developed out of proto-versions of the protocol in which the molecule was identified. For a fascinating historical perspective see (Matlin 2020).

“Empirical criteria” could be understood to supplement Feest’s account. They mediate the epistemic relationship between surface and hidden phenomena. They provide the conditions that individuate models of kinesin motility (hidden phenomenon) along experimentally tractable lines, which is just to say that they indicate the kinds of data patterns (surface phenomena) expected to correspond to them.

From the point of view of the 1989-2002 empirical criteria which individuated HoH characterizations of the “hidden phenomenon”—processivity and coordinated head activity—the corresponding data patterns (surface phenomena) are of the sort generated in the single-molecule work done during the same time period and discussed in the first part of section II. For instance, the empirical criterion “processivity” is a supposed feature of the “hidden” phenomenon of HoH walking—one head attached to MT at all times—that is understood or expected to generate certain observable and characteristic microtubule movements in a gliding assay or bead movements in a bead assay (surface phenomena). If the molecule walks processively, researchers expect a microtubule in a gliding assay to observably (under video microscopy) glide for a prolonged period without diffusing away from the immobilized kinesin molecule.

The 1989-2002 single-molecule work represents the ingenuity of single-molecule scientists in exploring how to vary the basic design of the single-molecule assay such that it would display the data patterns expected if a single kinesin molecule walked processively and coordinated its heads. This work represents what Feest refers to as the “skill and validation” aspects of the process of “stabilizing” phenomena. This includes an “element of physical craftsmanship and . . . an element of cognitive judgment (being able to recognize that an experiment or instrument in fact works” (62). Single-molecule researchers displayed both in physically designing the assay’s variations and in judging that, if the molecules walks

processively and coordinates its heads, then in *this* variation of the assay *these* data should show up.

A significant number variations on the assay generated data patterns that “fit” with the HoH characterization as specified by the 1989-2002 empirical criteria. In Feest’s terms, the phenomenon had been “stabilized”—researchers had “(a) empirically identif[ied] a given phenomenon and (b) gradually came to agree that the phenomenon is indeed a stable and robust feature of the world” (59). While a limited consensus had been established, however, single-molecule researchers were laboring under the limitations of the empirical criteria under which the phenomenon of single-molecule kinesin motility was initially constituted. As a result, the single-molecule assay was denied access to what I referred to above as an *experimental dead-space* consisting of merely conceptually distinct HoH models between which the single-molecule motility assay could not adjudicate.¹⁹ Perhaps we could say, following Feest, that the phenomenon remained “unstable” to a degree proportional to the ignorance reflected in the experimental dead-space. In order to enhance the probative value of the single-molecule assay and grant it access to this dead-space, the phenomenon of HoH walking—initially constituted under a characterization specified in terms of processivity and coordinated head activity—had to be reconstituted such that torque generation became the primary empirical criteria individuating alternative characterizations of kinesin motility. In other words, in order to render the phenomenon more “stable,” researchers realized that they had to start at the foundations. The

¹⁹ For the purposes of this paper, I do not intend my term “experimental dead-space” to refer to anything other than the particular pre-2002 space of merely conceptually distinct motility models. However, I suspect that the term could refer to a general category that may be of broader utility in the philosophical analysis of scientific practice. For instance, Bogen and Woodward (1988) discuss “bubble-chamber” experiments in particle physics designed to detect “weak neutral currents.” The experimental results themselves were not definitive and researchers engaged in indirect argumentation for and against the existence of weak neutral currents that deployed data and methods coming from outside of the bubble-chamber experimental program. This may constitute another instance of an experimental dead-space. See Bogen and Woodward (1989) pgs. 228-230.

very empirical criteria under which the phenomenon had been initially constituted required renovation. In short, the phenomenon needed to be reconstituted.

As I have argued, the Inchworm Episode took place entirely within the context of an experimental program dedicated to characterizing, rather than explaining, the phenomenon of kinesin motility. Though I would perhaps quibble with some of her terminology and supplement her view with the notion of “empirical criteria,” the fact that the dynamics described in my presentation of the case can be well captured by Feest’s account of how phenomena get stabilized (rather than explained) helps us to appreciate that the Inchworm Episode did not take place within an explanatory program. This is of particular philosophical interest as standard philosophical models have it that explanatory considerations drive phenomenon reconstitution.

(Bechtel and Richardson 1993/2010)’s model of phenomenon reconstitution, for instance, was motivated by their case study of the “Mendelian trait.” Classically, the Mendelian trait was understood as a macroscopically observable phenotypic trait. Faced with the fact that patterns of phenotypic inheritance could not be explained in terms of single genes, as phenotypic traits are the products of many genes in a complex organization, researchers in the middle of the 20th century abandoned the phenotypic trait as the central Mendelian unit in favor of a unit at a lower level of mechanistic analysis, the *enzyme*. Thus, the explanandum phenomenon to be accounted for in terms of single genes was reconstituted, shifting it down from the phenotypic trait to the enzyme, in the effort to develop mechanistic accounts of gene action.

(Craver 2007) discusses a further way in which phenomena can be reconstituted in the context of seeking mechanistic explanations. According to Craver, phenomena can be reconstituted in the wake of researchers recognizing that they have committed one of two errors – the “lumping error” or the “splitting error.” Both errors require inquiry into the phenomenon to

have developed to a point at which researchers have both a characterization of the phenomenon and putative mechanistic explanations on the table. Scientists observe they have committed the splitting error when they recognize that they have erroneously thought that some phenomena of interest are due to two or more distinct types of mechanisms when, in fact, they are due to mechanisms of the same type. They may then reconstitute the phenomena such that where once they thought of them as two distinct phenomena underpinned by two distinct types of mechanisms, they now understand them as one phenomenon underwritten by a single mechanism-type. The lumping error, on the other hand, occurs when a particular phenomenon is thought to be generated by a single mechanism while, in fact, two distinct mechanisms underwrite the phenomenon. In light of recognizing this error, scientists may reconstitute the phenomenon, considering it now as two distinct phenomena.

(Kronfeldner 2015)'s model differs from both of the above. She describes how phenomenon reconstitution can result not only as a result of researchers gaining insight at the level of mechanism, but also by researchers moving up to a level of greater abstraction. To illustrate, a researcher interested in explaining a particular phenotypic trait of a particular person—their height, say—will be unable to do so as it is widely recognized that such traits are the result of complex interactions between an individual's genetic inheritance and their ontogenetic environment. This does not mean, however, that genes do not explain. By moving up to an explanandum phenomenon at a greater level of abstraction, e.g. average differences between the heights of males and females in a population, researchers can appeal for explanation to differences in genotype, ignoring the complexity introduced by gene-environment interactions. In this way, researchers can hold fast to a particular “causal factor” in terms of which they wish to pitch their explanations and constitute the phenomena to be explained accordingly.

All three models have it that phenomenon reconstitution is driven by explanatory considerations. The research on kinesin motility discussed throughout this paper, however, involves experimental work dedicated solely to characterizing (stabilizing) the phenomenon of kinesin movement. Developing mechanistic explanations of kinesin movement (not discussed) involves researchers determining how the energy released from ATP-hydrolysis occurring in the molecule's nucleotide binding sites results in characteristic structural changes throughout the molecule. Mechanistic explanation asks after the role played (if any) by thermal forces in bringing the heads forward in their stepping pattern. It attempts to determine whether elastic tension on the neck linker generated as the molecule stretches during its walk provides energy—in addition to that provided by ATP-hydrolysis—that may or may not be necessary for walking. These (and further issues) are, of course, important for developing mechanistic explanations for kinesin motility—for answering the question of *by what means* kinesin manages to walk in the way it does. But considerations at this explanatory level did not, as we saw, figure into the reconstitution story. Again, it took place entirely within the context of experimental efforts to characterize the phenomenon—to characterize the way kinesin walks, not the means by which it manages to walk that way.

Colaço (2020) notes “there is a lacuna in the literature regarding how researchers determine whether their characterization of a target phenomenon is appropriate for their aims” (1). Colaço helps illuminate this lacuna, using the case of LTP research to show the way in which our understanding of phenomena should be revised that do not depend on explanation. My analysis of the “inchworm episode” sheds further light. In order to experimentally adjudicate between alternative characterizations of kinesin motility, single-molecule researchers sought *empirical* criteria by which to individuate them—criteria that distinguished them along lines that

were testable from the point of view of the single-molecule motility assay. It was determined that individuating characterizations of kinesin motility by appeal to torque generation rather than merely processivity and coordinated head activity, enabled access to what was antecedently an experimental dead-space consisting of merely conceptually distinct motility models. The new taxonomy rendered that space experimentally accessible to the single-molecule motility assay. Thus, the Inchworm Episode illustrates how researchers can recharacterize—or, better, reconstitute—phenomena to the end of enhancing the probative value of their experimental tools.

Acknowledgments: Thanks to the UCSD Philosophy of Science and Practice Reading Group – especially Richard Vagnino, Linus Huang and Gina Dandreapenna. Thanks also to Juan-Carlos Gonzales and Adam Fox for many helpful conversations. Special thanks to William Bechtel for reading numerous drafts of the manuscript and providing sage advice throughout its development.

*The author declares that they have no conflict of interest.

References

Asbury, C. L., Fehr, A. N., & Block, S. M. (2003). Kinesin moves by an asymmetric hand-over-hand mechanism. *Science*, 302(5653), 2130-2134.

Bechtel, W., & Richardson, R. C. (2010). *Discovering complexity: Decomposition and localization as strategies in scientific research*. MIT press. *philosophical perspectives on cognitive neuroscience*.

Bechtel, W., & Abrahamsen, A. (2005). Explanation: A mechanist alternative. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, 36(2), 421-441.

Berliner, Elise, Edgar C. Young, Karin Anderson, Hansraj K. Mahtani, and Jeff Gelles. "Failure of a single-headed kinesin to track parallel to microtubule protofilaments." *Nature* 373, no. 6516 (1995): 718-721.

Bickel, J. (2003) *Philosophy and Neuroscience: A Ruthlessly Reductive Approach*. Dordrecht: Kluwer (now Springer) Academic Publishers.

Bickle, J. (2016). Revolutions in neuroscience: Tool development. *Frontiers in systems neuroscience*, 10, 24.

Block, S. M. (1998). Kinesin: what gives?. *Cell*, 93(1), 5-8.

Block, S. M., Goldstein, L. S., & Schnapp, B. J. (1990). Bead movement by single kinesin molecules studied with optical tweezers. *Nature*, 348(6299), 348.

Block, S. M., & Svoboda, K. (1995). Analysis of high resolution recordings of motor movement. *Biophysical journal*, 68(4 Suppl), 237s.

Bloom, G. S., Wagner, M. C., Pfister, K. K., & Brady, S. T. (1988). Native structure and physical properties of bovine brain kinesin and identification of the ATP-binding subunit polypeptide. *Biochemistry*, 27(9), 3409-3416.

Bogen, J., & Woodward, J. (1988). Saving the phenomena. *The Philosophical Review*, 97(3), 303-352..

Colaço, D. (2018). Rip it up and start again: The rejection of a characterization of a phenomenon. *Studies in History and Philosophy of Science Part A*, 72, 32-40.

Colaço, D. (2020). Recharacterizing scientific phenomena. *Euro Jnl Phil Sci* 10, 14, <https://doi.org/10.1007/s13194-020-0279-z>

Craver, C. F. (2002). Interlevel experiments and multilevel mechanisms in the neuroscience of memory. *Philosophy of Science*, 69(S3), S83-S97.

Craver, C. F. (2007). *Explaining the brain: Mechanisms and the mosaic unity of neuroscience*. Oxford University Press.

Craver, Carl and James Tabery, "Mechanisms in Science", *The Stanford Encyclopedia of Philosophy* (Summer 2019 Edition), Edward N. Zalta (ed.), URL = [<https://plato.stanford.edu/archives/sum2019/entries/science-mechanisms/>](https://plato.stanford.edu/archives/sum2019/entries/science-mechanisms/).

Cross, R. A. (1995). On the hand over hand footsteps of kinesin heads. *Journal of muscle research and cell motility*, 16(2), 91-94.

Duhem, P. (1969). *To Save the Phenomena: An Essay on the Idea of Physical Theory from Plato to Galileo*, trans. E. Doland and C. Maschler (Chicago, 1969).

Feest, U. (2010). Concepts as tools in the experimental generation of knowledge in cognitive neuropsychology. *Spontaneous Generations: A Journal for the History and Philosophy of Science*, 4(1), 173-190.

Feest, U. (2011). What exactly is stabilized when phenomena are stabilized?. *Synthese*, 182(1), 57-71

Feest, U. (2017). Phenomena and objects of research in the cognitive and behavioral sciences. *Philosophy of Science*, 84(5), 1165-1176.

Hancock, W. O., & Howard, J. (1998). Processivity of the motor protein kinesin requires two heads. *The Journal of cell biology*, 140(6), 1395.

Harrison, B. C., Marchese-Ragona, S. P., Gilbert, S. P., Cheng, N., Steven, A. C., & Johnson, K. A. (1993). Decoration of the microtubule surface by one kinesin head per tubulin heterodimer. *Nature*, 362(6415), 73.

Higuchi, H., Bronner, C. E., Park, H. W., & Endow, S. A. (2004). Rapid double 8-nm steps by a kinesin mutant. *The EMBO journal*, 23(15), 2993-2999.

Hoenger, A., Thormaehlen, M., Diaz-Avalos, R., Doerhoefer, M., Goldie, K. N., MuÈller, J., & Mandelkow, E. (2000). A new look at the microtubule binding patterns of dimeric kinesins. *Journal of molecular biology*, 297(5), 1087-1103.

Howard, J. (1996). The movement of kinesin along microtubules. *Annual review of physiology*, 58(1), 703-729.

Howard, J., Hudspeth, A. J., & Vale, R. D. (1989). Movement of microtubules by single kinesin molecules. *Nature*, 342(6246), 154.

Hua, W., Chung, J., & Gelles, J. (2002). Distinguishing inchworm and hand-over-hand processive kinesin movement by neck rotation measurements. *Science*, 295(5556), 844-848.

Hunt, A. J., & Howard, J. (1993). Kinesin swivels to permit microtubule movement in any direction. *Proceedings of the National Academy of Sciences*, 90(24), 11653-11657.

Illari, P. M., & Williamson, J. (2012). What is a mechanism? Thinking about mechanisms across the sciences. *European Journal for Philosophy of Science*, 2(1), 119-135.

Kaseda, K., Higuchi, H., & Hirose, K. (2003). Alternate fast and slow stepping of a heterodimeric kinesin molecule. *Nature Cell Biology*, 5(12), 1079.

Kikkawa, M., Ishikawa, T., Nakata, T., Wakabayashi, T., & Hirokawa, N. (1994). Direct visualization of the microtubule lattice seam both in vitro and in vivo. *The Journal of cell biology*, 127(6), 1965-1971.

Kronfeldner, M. (2015). Reconstituting phenomena. In *Recent Developments in the Philosophy of Science: EPSA13 Helsinki* (pp. 169-181). Springer, Cham.

Machamer, P., Darden, L., & Craver, C. F. (2000). Thinking about mechanisms. *Philosophy of science*, 67(1), 1-25.

Matlin, K. S. (2020). Microscopes and Moving Molecules: The Discovery of Kinesin at the Marine Biological Laboratory. In Matlin, K. S., Maienschein, J., & Ankeny, R. A. (Eds.). (2020). *Why Study Biology by the Sea?*. University of Chicago Press. 211.

Ray, S., Meyhöfer, E., Milligan, R. A., & Howard, J. (1993). Kinesin follows the microtubule's protofilament axis. *The Journal of cell biology*, 121(5), 1083-1093.

Scholey, J. M., Heuser, J., Yang, J. T., & Goldstein, L. S. (1989). Identification of globular mechanochemical heads of kinesin. *Nature*, 338(6213), 355.

Song, Young-Hwa, and Eckhard Mandelkow. "Recombinant kinesin motor domain binds to beta-tubulin and decorates microtubules with a B surface lattice." *Proceedings of the National Academy of Sciences* 90, no. 5 (1993): 1671-1675.

Song, Y. H., & Mandelkow, E. (1995). The anatomy of flagellar microtubules: polarity, seam, junctions, and lattice. *The Journal of cell biology*, 128(1), 81-94.

Sullivan, J. A. (2009). The multiplicity of experimental protocols: a challenge to reductionist and non-reductionist models of the unity of neuroscience. *Synthese*, 167(3), 511.

Svoboda, K., Schmidt, C. F., Schnapp, B. J., & Block, S. M. (1993). Direct observation of kinesin stepping by optical trapping interferometry. *Nature*, 365(6448), 721.

Tucker, Carla, and Lawrence SB Goldstein. "Probing the kinesin-microtubule interaction." *Journal of Biological Chemistry* 272, no. 14 (1997): 9481-9488.

Vale, R. D., Reese, T. S., & Sheetz, M. P. (1985). Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*, 42(1), 39-50.

Vale, Ronald D., Takashi Funatsu, Daniel W. Pierce, Laura Romberg, Yoshie Harada, and Toshio Yanagida. "Direct observation of single kinesin molecules moving along microtubules." *Nature* 380, no. 6573 (1996): 451-453.

Van Fraassen, B. C. (1980). *The scientific image*. Oxford University Press.

Walker, R. A. "Ncd and kinesin motor domains interact with both alpha-and beta-tubulin." *Proceedings of the National Academy of Sciences* 92, no. 13 (1995): 5960-5964.

Yildiz, A., Tomishige, M., Vale, R. D., & Selvin, P. R. (2004). Kinesin walks hand-over-hand. *Science*, 303(5658), 676-678.