



Video article

A strikingly-angled spindle mediates nuclear migration during colonization of rice cells infected by *Magnaporthe oryzae*

Mariel A. Pfeifer¹, Kiersun Jones¹, Chang Hyun Khang^{*}

Department of Plant Biology, University of Georgia, Athens, GA 30602, USA



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ABSTRACT

To cause rice blast disease, *Magnaporthe oryzae* must properly organize microtubules and position nuclei during colonization of host cells. Live cell confocal imaging of fluorescently-tagged microtubules and nuclei of *M. oryzae* invasive hyphae reveals that microtubules form a cage-like arrangement around nuclei during interphase and that the mitotic spindle forms and mediates nuclear migration while integrity of the nuclear envelope is lost. Our results also unveil a strikingly-angled spindle during nuclear migration through the narrow invasive hyphal peg, suggesting a yet-to-be discovered mechanism of mitotic nuclear migration when invasive hyphae move to adjacent rice cells.

1. Introduction

Magnaporthe oryzae is a destructive fungal pathogen, causing blast disease in rice and other economically significant plants. To cause disease, *M. oryzae* develops a specialized penetration cell, called the appressorium, which can deliver multiple daughter nuclei to invasive hyphae (IH) growing inside rice cells (Jenkinson et al., 2017). The fungus uses a form of intermediate mitosis to proliferate within the first-invaded rice cell (Jones et al., 2016; Pfeifer and Khang, 2018; Shipman et al., 2017). After colonization of the first-invaded cell, IH begin cell-to-cell movement by scanning the rice cell wall presumably searching for suitable crossing points located in pitfields (Kankanala et al., 2007; Sakulkoo et al., 2018). Once a crossing point is identified, *M. oryzae* develops an extremely constricted structure, called the IH peg (~0.5 µm), which serves as a conduit connecting a mother IH cell to a daughter IH cell located in a newly-invaded rice cell (Kankanala et al., 2007). In our previous studies of IH cell-to-cell movement, a single nucleus divided in the mother IH cell, and one daughter nucleus elongated to successfully cross the narrow IH peg by an unknown mechanism (Jones et al., 2016).

In fungi, microtubules (MTs) play significant roles mediating nuclear migration throughout the cell cycle (Xiang, 2017). In *M. oryzae*, MT arrangement and behavior during vegetative growth and appressorium development are characterized, but little is known about MT dynamics during colonization of host cells (Czymmek et al., 2005; Row

et al., 1985; Saunders et al., 2010a, 2010b; Veneault-Fourrey et al., 2006). Here, we report MT dynamics in IH located in the first-invaded cell and during cell-to-cell movement. We demonstrate that nucleation of incipient IH cells occurs via mitotic nuclear migration, and that spindle choreography is complex during nuclear migration through the IH peg.

2. Results and discussion

2.1. Interphase arrangements of microtubules during rice infection

We created *M. oryzae* strain CKF3578 by introducing β-tubulin-GFP, labelling MTs (Freitag et al., 2004), into an *M. oryzae* strain coexpressing histone H1 fused to tdTomato (H1-tdTomato) and tdTomato fused to a nuclear localization signal (tdTomato-NLS). H1-tdTomato and tdTomato-NLS colocalize within the nucleus in interphase, but at the start of mitosis tdTomato-NLS disperses from the nucleus into the cytoplasm, as similarly shown with the *M. oryzae* mitotic reporter strain expressing GFP-NLS and H1-tdTomato (Jones et al., 2016). Thus, *M. oryzae* CKF3578 permits study of MTs in the context of the *M. oryzae* cell cycle, with dispersal of tdTomato-NLS from the nucleus serving as an indicator of loss of nuclear envelope integrity (Pfeifer and Khang, 2018). Confocal microscopy of CKF3578 growing inside rice cells revealed that MTs are typically positioned along the growth axis and follow the curvature of IH cells during interphase (Fig. 1A). In many

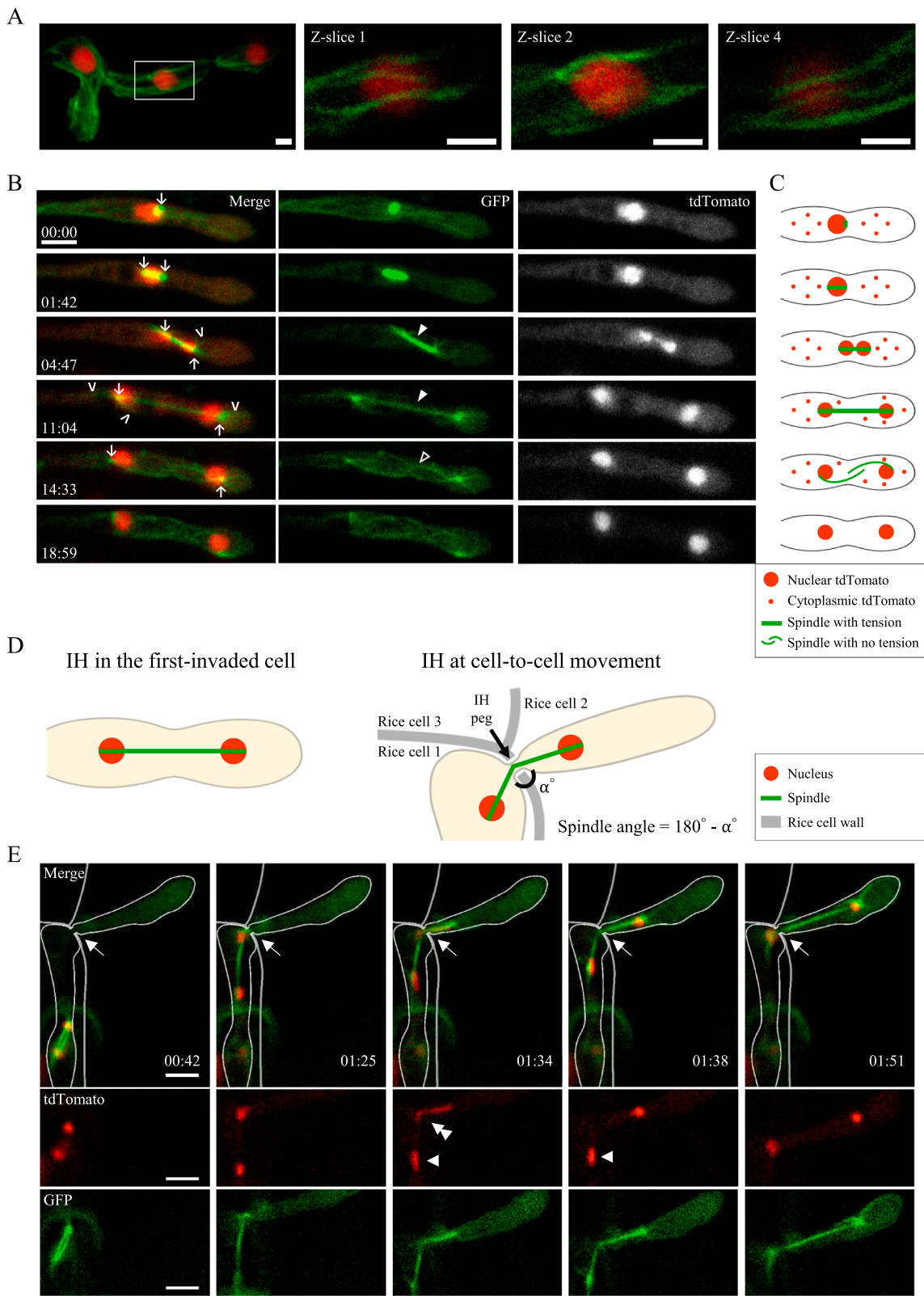
^{*} Corresponding author.

E-mail address: ckhang@uga.edu (C.H. Khang).

¹ These authors contributed equally to this work.

fungi, such as *Neurospora crassa* and *Saccharomyces cerevisiae*, MTs are nucleated from microtubule-organizing centers (MTOCs) associated with the nuclear envelope called spindle pole bodies (SPBs) during interphase (Kilmartin and Adams, 1984; Roca et al., 2010). In contrast, the cytoplasmic MTs we observe during interphase in *M. oryzae* do not appear to nucleate from a SPB and instead form a cage-like

arrangement around the nucleus (Fig. 1A). This is similar to patterns of SPB-independent MTs observed in the yeast-like growth of *Ustilago maydis* (Straube et al., 2005) and suggests that *M. oryzae* utilizes MTOCs other than SPBs during interphase.



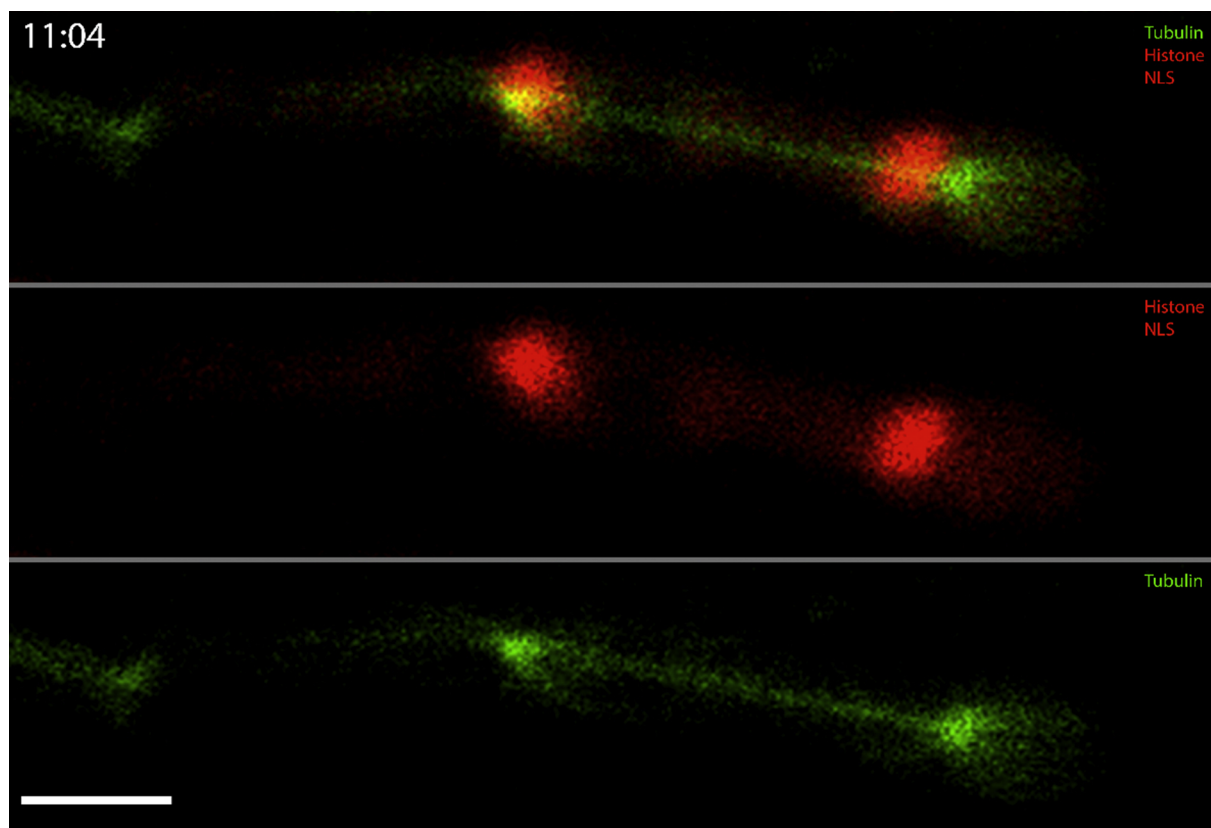
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Fig. 1. Microtubule (MT) and nuclear dynamics of *M. oryzae* strain CKF3578 during rice infection. CKF3578 expresses β -tubulin-GFP (green), H1-tdTomato (red), and tdTomato-NLS (red). Note that H1-tdTomato is associated with DNA throughout the cell cycle whereas tdTomato-NLS is localized in nuclei during interphase but dispersed in the cytoplasm during mitosis. (A) Confocal images revealing MT arrangement during interphase of *M. oryzae* invasive hyphae (IH) in the first-invaded rice cell. Interphase is indicated by absence of tdTomato-NLS in the cytoplasm. MTs are arranged along the growth axis and follow the curvature of IH. The left panel shows a maximum intensity projection of four z-slices, spanning 4 μ m. The white box denotes a cage-like arrangement of MTs around the nucleus and is shown in detail in the second through fourth panels, which are at different focal planes. In relation to z-slice 1, z-slice 2 is 1 μ m below, and z-slice 4 is 3 μ m below. Bars = 2.5 μ m. (B) Time-lapse confocal images revealing MT and nuclear dynamics during mitosis in leading IH in the first-invaded rice cell. The images included are select still images from [Video 1](#) shown as merged fluorescence (left), β -tubulin-GFP (middle) and H1-tdTomato/tdTomato-NLS (pseudocolored white; right). In the merged panels, arrows show spindle pole bodies, and arrowheads denote noticeable astral microtubules. In the GFP panels, solid arrowheads show tension within the spindle (04:47 and 11:04), and loss of spindle tension is shown by an open arrowhead (14:33). Cytoplasmic tdTomato (tdTomato-NLS) is already dispersed from the nucleus at 00:00 and is fully reimported into the nucleus in the bottom panel (18:59). Times are in minutes: seconds. Bar = 5 μ m. (C) A schematic representation of tdTomato localization relative to spindle dynamics during mitosis in leading IH in the first-invaded rice cell. (D) A schematic representation of nuclear (red) and spindle (green) positioning in leading IH located in the first-invaded rice cell (left) and during hyphal movement from Rice cell 1 to Rice cell 2 (right). (E) Time-lapse confocal images revealing spindle and nuclear dynamics during hyphal cell-to-cell movement through the IH peg as represented in (D) right. The images included are select still images from [Video 2](#). Images are shown in the top panel (merge). tdTomato fluorescence (middle) shows nuclear dynamics. tdTomato-NLS signal is faintly observed in the cytoplasm throughout the time-series, indicating mitosis. GFP fluorescence (bottom) shows the spindle. Arrows indicate the IH peg. Single arrowheads show a slightly elongated nucleus, and a double arrowhead shows a stretched nucleus migrating through the IH peg. As the spindle migrates through the IH peg, it adopts an angle of 66° (01:34). Bars = 5 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Mitotic dynamics during IH growth inside the first-invaded rice cell

Using time-lapse confocal microscopy of *M. oryzae* CKF3578, we determined mitotic MT dynamics in the most apical, or leading, IH cell growing inside the first-invaded rice cell. A representative example is shown in [Video 1](#), with selected single-frames from [Video 1](#) included in [Fig. 1B](#). We first identified an IH cell in prophase based on the presence of a single nucleus with tdTomato-NLS dispersed in the cytoplasm and a bright focus of β -tubulin-GFP at the edge of the nucleus, presumably indicating duplicated SPBs ([Video 1](#), [Fig. 1B](#); 00:00). At this stage, interphase cytoplasmic MTs are visible but begin disassembling. We observe SPBs migrate to opposite sides of the nucleus (01:42). As the tdTomato-tagged chromosomes separate, astral MTs appear and form transient associations with the cell cortex, behaving dynamically and increasing in average length (04:47). Oscillatory movements of the spindle (e.g., multiple reversals in migration direction), combined with

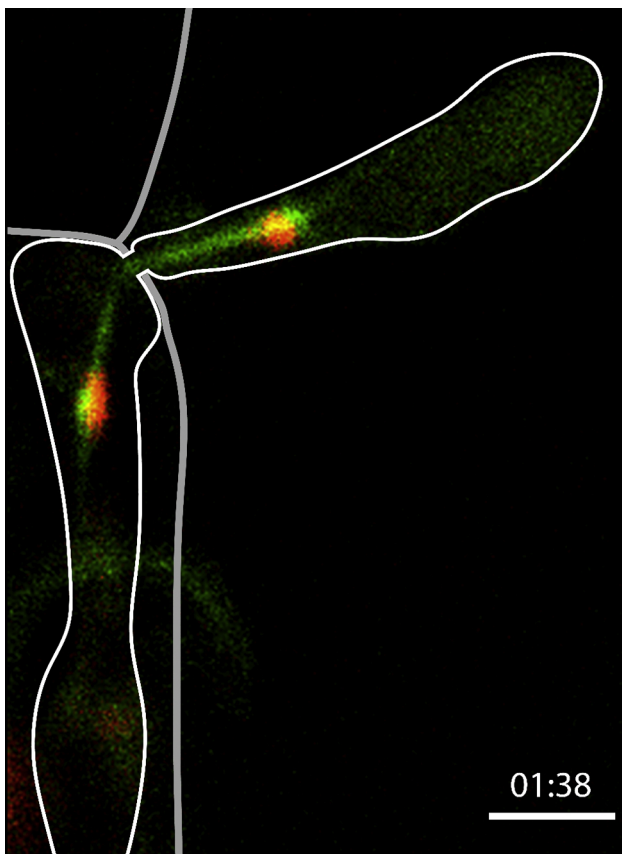
spindle extension, result in the net movement of the daughter nucleus closer to the incipient IH cell ([Video 1](#)). Once the daughter nucleus approaches its interphase position in the incipient IH cell, the spindle reaches a maximum length (11:04). At this stage, the spindle appears as a straight-line, which we interpret as tension within the spindle (11:04). As the spindle disassembles, tension in the spindle is lost, and the spindle appears to be unraveled (14:33). Subsequently, interphase cytoplasmic MTs reappear, and tdTomato-NLS is reimported into the nucleus (18:59). Our results indicate that the spindle forms and mediates nuclear migration while compartmentalization between the nucleus and the cytoplasm is lost ([Fig. 1C](#)). This suggests increased permeability of the nuclear envelope during mitosis and supports our earlier hypothesis that *M. oryzae* uses intermediate mitosis during rice infection ([Jenkinson et al., 2017](#); [Jones et al., 2016](#); [Pfeifer and Khang, 2018](#); [Shipman et al., 2017](#)). Our results also show that spindles are straightly arranged when IH colonize the first-invaded rice cell ([Fig. 1B](#) and [D](#) left).



Supplementary Video 1.

2.3. During nuclear movement through the IH peg, the spindle adopts an extremely angled morphology

Using the same time-lapse approach as we did in the first-invaded cell, we examined the dynamics of MTs during nuclear migration through the IH peg (Fig. 1D right, Video 2, and selected frames in Fig. 1E). The example shown in Video 2 is representative, and select still images from Video 2 are presented in Fig. 1E. In early anaphase, the interpolar MTs of the spindle appear as bars connecting separated chromosomes (Fig. 1E, 00:42). Prior to nuclear migration through the IH peg, the spindle is aligned with the growth axis of the mother IH cell in Rice cell 1 (Fig. 1D right and Fig. 1E 01:25). During nuclear migration through the IH peg, the nucleus stretches, and the spindle is noticeably angled (Fig. 1E, 01:34 and 01:38). Once the migrating nucleus crosses the IH peg, it again becomes spherical (01:38). The spindle continues to elongate in an angular-manner (01:38). Once maximum length of the spindle is achieved (01:51), a sudden loss of tension in the spindle occurs, marking the start of spindle disassembly (not shown in video). In all observations ($n = 10$), the spindle persists with obvious tension between the two SPBs before, during, and after nuclear migration through the IH peg, indicating that nuclear migration through the IH peg occurs during mitosis. Intriguingly, we also observe the spindle displaying distinct choreography as the nucleus crosses the IH peg (Video 2; Fig. 1E, 01:34 and 01:38). In 80% of nuclear migration events through the IH peg, the spindle becomes strikingly angled, ranging from 30° to 74° (52° mean $\pm 14^\circ$). The spindle in the remaining 20% of events appears less-angled and more curved. Compared to other fungi, e.g., *Schizosaccharomyces pombe* and *Saccharomyces uvarum*, the angled spindle during migration through the IH peg in *M. oryzae* is extreme (Kilmartin and Adams, 1984; Tanaka and Kanbe, 1986). Such angled spindle morphology is likely a function of the unique shape of IH cells at the cell-to-cell movement stage of rice infection. It is common to observe these IH displaying extensively curved or angled geometries compared to leading IH. Given the link between cell geometry and spindle alignment (Daga and Nurse, 2008), we propose that the spindle requires special coordination to navigate the IH peg and deliver the daughter nucleus to the incipient IH cell.



Supplementary Video 2.

In sum, our results provide novel insight about mitosis and nuclear migration of *M. oryzae* during rice infection. We show that during interphase in IH cells, MTs form a cage-like arrangement around the nucleus. Prior to spindle formation, compartmentalization between the nucleus and cytoplasm is lost, a hallmark of intermediate mitosis (Pfeifer and Khang, 2018). Spindle dynamics demonstrate that nucleation of incipient IH within the first-invaded cell and at the cell-to-cell movement stage of infection occurs via mitotic nuclear migration. Remarkably, a majority of spindles adopt drastic angles during nuclear migration through the confined IH peg. These observations suggest that mechanisms of spindle positioning and alignment are especially important for proper nuclear migration at the cell-to-cell movement stage of rice infection. Discovering the key molecular players in the underlying mechanism may lead to identification of targets for antifungals in the future, providing a means to block fungal proliferation beyond the first-invaded host cell.

3. Methods

M. oryzae wild-type strain O-137 was transformed sequentially with two binary vectors pCK1528 and pCK1728 to generate transformant CKF3578 using Agrobacterium-mediated transformation (Khang et al., 2005). pCK1528 was produced by cloning NLS (three tandem repeats of the nuclear localization signal from simian virus large T-antigen) at the C terminus of tdTomato under control of the *M. oryzae* ribosomal protein 27 gene (RP27) promoter in binary vector pBGt (G418 selection; Kim et al., 2011). pCK1728 was produced by cloning histone H1 at the 5' end of tdTomato under control of the RP27 promoter (Shipman et al., 2017) and β -tubulin at the 5' end of GFP under control of *Neurospora* ccg-1 promoter from pMF309 (Freitag et al., 2004) in binary vector pBHT2 (hygromycin selection). Rice variety YT16 was grown and inoculated as previously described (Jones and Khang, 2018).

Confocal microscopy was performed on a Zeiss 880 confocal system using a Plan-Neofluor $40\times/1.3$ NA (oil) objective. Excitation/emission wavelengths were 488 nm/505–530 nm (GFP), and 543 nm/560–615 nm (tdTomato). Images were analyzed and processed using a combination of the Zen software (Black edition), Adobe Photoshop, and ImageJ (<http://imagej.nih.gov/ij/>). Spindle angles were measured with the angle tool in ImageJ. Angle measurements were transformed as described in Fig. 1D and analyzed using JMP Pro Version 13.2.

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