Figure S1 (supplement for Figure 2). The effect of imaging duration, temperature and using mounting pads molded with a vinyl LP record on GSC mitosis. (A) Beeswarm plots overlaid with boxplots showing the number of mitotic entries per gonad (top) and the per cell duration of congression (bottom) for the control data used in Figure 2. Data were binned by 10-minute intervals spanning the 90-minute acquisition. Box edges are the 25th and 75th percentiles, whiskers extend to the furthest data points, outliers are indicated by a ‘+'. Data were compared using a Kruskal-Wallis test with a Tukey-Kramer post hoc test. Bins that are significantly different from the first, 0 to 10-minute bin are noted. The number of mitotic entries decreases significantly by 50 minutes of imaging. Statistically significant increases in the duration of congression are observed in some bins after 40 minutes of imaging. Only cells that undergo a complete congression within each 10-minute interval were considered, which excludes any cells with a duration of congression > 10 minutes. (B-C) Beeswarm plots showing the number of mitotic entries per gonad (left) and the per cell and per gonad mean duration of congression (right) in (B) animals mounted on 3% agarose pads molded with grooves from a vinyl LP record (blue; n = 13 animals, 154
cells) compared to animals mounted under standard conditions and in (C) in animals raised at 20°C and imaged at 15°C (purple; n = 7 animals, 47 cells), 20°C (yellow; n = 5 animals, 37 cells) and 25°C (magenta, n=6 animals, 53 cells). Control data in B (grey) is reproduced from Figure 2. For B and C, black bars represent the mean of the per animal (left) and per cell (right) values; error bars represent the standard deviation. In (B), data were compared using a two-tailed Student's t-test. Data in (C) were compared using a Kruskal-Wallis test with a Tukey-Kramer post hoc test. All significant differences (p > 0.05) are noted. For all panels, per gonad means are represented by diamonds and per cell values by circles. ns = not significant, p ≥ 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001 *** = p < 0.001.
Figure S2 (supplement for Figure 3). Kymographs showing a line scan through the spindle of three GSCs from different gonads over time, before (left) and after (right) x-y registration (CentTracker Module 1). Pre-registration, centrosome pairs move in tandem with sample movement, and the magnitude of this ‘global’ movement is greater than the movement of individual centrosomes during spindle assembly. Registration eliminates the majority of sample movement such that centrosome movements during spindle assembly become obvious. The approximate time of NEBD, congression start and end and peak spindle elongation during anaphase are noted by colored dots (right). Kymographs were generated using the KymographBuilder plugin in ImageJ (Hadrien et al., 2016).
**Figure S3 (supplement for Figure 4).** PCA and hierarchical clustering of GSC mitotic features does not reveal natural clustering. (A) Bar graph combined with cumulative distribution showing the percent of variance explained by each of the possible 34 principle components (PCs). The first three PCs (boxed in yellow) explain < 50% of the variance. (B) Plot showing the silhouette coefficients for all observations (here each a GSC) sorted by cluster, as determined by *k*-means clustering. The silhouette coefficient represents how similar an observation is to its own cluster, relative to the nearest neighboring cluster, with similarity calculated using Euclidean distance, and is a measurement of how well a given observation fits within its assigned cluster. The mean silhouette score is shown in red. (C-D) PCA plots for 547 GSCs plotted along the first 3 PCs and colored-coded according to their time of mitotic entry (NEBD) relative to the start of image acquisition (C) or their position in the mitotic zone relative to the distal tip of the gonad (D). (E) Heat map showing the normalized values for all 34 mitotic features (listed on the left), plus the time of mitotic entry (NEBD) relative to the start of image acquisition, for GSCs from each strain of origin (top), and ordered by hierarchical clustering, with the linkage between clusters indicated by the dendrogram to the right. Dendrogram branch length represents the Euclidean distance between clusters.
Figure S4 (supplement for Figure 5). GSC spindles also preferentially orient along the gonadal D/P axis in 1-day old adults. (A) Schematic representation of the ~20 cell diameters (100 µm)-long mitotic zone of an adult gonad (based on Crittenden et al., 2006), with the distal tip (magenta circle) and distal-proximal (D/P) axis (magenta line) indicated. The morphology of both the DTC and Sh1 elaborates between the L4 and adult stage and the uneven DTC-Sh1 interface spans a region approximately 20-40 µm from the distal tip (Gordon et al., 2020). (B) Histogram showing the number of GSC mitoses along the D/P axis in 1-day old adults, in 10 µm/~2 cell diameter bins. Grey bars represent the total number of mitoses for each bin. Purple line shows the normal distribution fit. Mean ± SD = 40.18 ± 21.07 µm. n = 147 cells from 19 animals. (C) The cumulative distribution for anaphase spindle angles, relative to the D/P axis, for L4 larvae (magenta) and 1-day old adults (purple), as compared to the calculated random distribution (yellow),
with the corresponding polar histograms for late L4 larvae and adults shown below. Anaphase spindles in 1-day old adults are enriched for horizontal angles, i.e. towards the D/P axis, compared to random \( (p = 3.2 \times 10^{-6}, \text{one-sample Kolmogorov-Smirnov test}) \), but are less biased than anaphase spindles in L4 larvae \( (\lambda_p = 0.49 \text{ in adults versus } \lambda_p = 0.79 \text{ in L4 larvae}; p = 0.011; \text{two-sample Kolmogorov-Smirnov test}) \). L4 data are reproduced from Figure 5B-C. (D) Polar histograms showing the distribution of anaphase spindle angles in 1-day old adults, relative to the D/P axis, binned by distance from the gonad’s distal tip. Bin edges and n are given underneath. Bin sizes were selected as for L4 larvae to correspond to the approximate DTC-only, DTC-Sh1 interface and Sh1-only regions (Gordon et al., 2020) and following the distribution of mitoses such that the middle 20-50 \( \mu \)m encompasses the peak zone of proliferation. The distribution of angles is similar in all bins \( (p > 0.05; \text{two-sample Kolmogorov-Smirnov test}) \) and significantly different from random \( (\lambda_p > 0; p = 0.011, p = 0.02 \text{ and } p = 0.002, \text{respectively}; \text{one-sample Kolmogorov-Smirnov test}) \).
**Table S1.** Descriptions of the mitotic features extracted from centrosome tracking and spindle length measurements in GSCs. Features were measured for NEBD, congression and anaphase as follows: NEBD values were calculated over 3 timepoints, the user-defined NEBD and the two preceding timepoints, except for the duration of NEBD, which is the amount of time between the timepoint marked as NEBD and the calculated start of congression. Congression values were calculated over all timepoints within the congression period. Anaphase values fall into two categories: *early anaphase* values were calculated over 5 timepoints, the last timepoint of congression and the following 4 timepoints (2 minutes), which correspond to the period of rapid spindle elongation; *anaphase* values were calculated over 9 timepoints, the last time point of congression and the following 8 timepoints (4 minutes), at which point spindle elongation is largely complete. Where defined, the distal and proximal centrosomes were the centrosome closest to and farthest from the distal tip of the gonad, respectively.

| Feature                                           | Description                                                                                                                                                                                                 |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell distance from distal tip along D/P axis      | The mean spindle midpoint position along the D/P axis over all timepoints analyzed                                                                                                                       |
| Time NEBD relative to acquisition start           | The timepoint at which a cell enters mitosis (NEBD) relative to the start of image acquisition.                                                                                                             |
| Duration (NEBD)                                   | The amount of time (minutes) between NEBD and the calculated start of congression                                                                                                                        |
| Duration (congression)                            | The amount of time (minutes) that a cell spends in congression, as defined in the Methods section.                                                                                                        |
| Centrosome separation (NEBD)                      | The distance between centrosomes at NEBD, calculated as the mean centrosome-to-centrosome distance at NEBD and the 2 prior timepoints.                                                                    |
| Mean spindle length (congression)                 | The mean centrosome-to-centrosome distance during congression.                                                                                                                                           |
| Mean spindle length fluctuation (congression)     | The mean timepoint-to-timepoint change in spindle length during congression.                                                                                                                             |
| Mean change in spindle midpoint position (congression) | The mean timepoint-to-timepoint displacement (Euclidean distance in 3D between timepoints) of the spindle midpoint during congression.                                                                    |
| Max. anaphase spindle length                      | The spindle length after spindle elongation is largely complete (4 minutes after anaphase onset).                                                                                                         |
| Spindle elongation rate (anaphase)                | Mean increase in spindle length over time during early anaphase.                                                                                                                                          |
| Angle to D/P axis (NEBD, congression and anaphase) | The mean angle of intersection between the spindle and the D/P axis                                                                                                                                     |
| Mean change in spindle orientation to D/P axis    | The mean timepoint-to-timepoint change in the angle of intersection between the spindle and the D/P axis.                                                                                                 |
| (congression and anaphase) | Spindle rotational range around D/P axis (congression and anaphase) |
|---------------------------|----------------------------------|
|                           | The maximum angle of intersection between the spindle and the D/P axis. Represents the range of rotation around the D/P axis that each spindle explores. |

| Mean spindle angular displacement (congression and anaphase) | Mean spindle angular displacement (congression and anaphase) |
|-------------------------------------------------------------|-------------------------------------------------------------|
|                                                             | The mean timepoint-to-timepoint change in spindle angle, relative to itself (i.e. in space). |

| Max. spindle angular displacement (congression and anaphase) | Max. spindle angular displacement (congression and anaphase) |
|-------------------------------------------------------------|-------------------------------------------------------------|
|                                                             | The largest difference in spindle angle, relative to itself. Represents the maximum range of rotation in space that each spindle explores. |

| Mean centrosome arc length (congression and anaphase) | Mean centrosome arc length (congression and anaphase) |
|-------------------------------------------------------|-------------------------------------------------------|
|                                                       | The mean timepoint-to-timepoint arc length (s) for centrosomes. Assuming that the spindle rotates around its midpoint, such that the radius (r) of the sphere being traversed by the centrosomes at a given timepoint = spindle length/2, $s = \frac{\pi r \theta}{180^\circ}$. Spindle length = the mean measured length between two timepoints. $\theta$ = angular displacement between timepoints. |

| Anaphase displacement (centrosome) | Anaphase displacement (centrosome) |
|-----------------------------------|-----------------------------------|
|                                   | The Euclidean distance in 3D between the position of each centrosome at anaphase onset and its position after spindle elongation is largely complete (4 minutes later). |

| Anaphase velocity (centrosome) | Anaphase velocity (centrosome) |
|---------------------------------|---------------------------------|
|                                 | The mean velocity, in 3D, of each centrosome in early anaphase. |

| Anaphase displacement along D/P axis (centrosome) | Anaphase displacement along D/P axis (centrosome) |
|--------------------------------------------------|--------------------------------------------------|
|                                                 | The projected distance in x, along the D/P axis, between the position of each centrosome at anaphase onset and its position after spindle elongation is largely complete (4 minutes later). |

| Anaphase velocity along D/P axis (centrosome) | Anaphase velocity along D/P axis (centrosome) |
|-----------------------------------------------|-----------------------------------------------|
|                                               | The mean velocity of each centrosome along the D/P axis in early anaphase. |

| Dif. in anaphase displacement (distal - proximal centrosome) | Dif. in anaphase displacement (distal - proximal centrosome) |
|-------------------------------------------------------------|-------------------------------------------------------------|
|                                                             | The difference in anaphase displacement between the distal and proximal centrosomes. Positive values indicate a greater displacement for the distal centrosome, etc. |

| Dif. in anaphase velocity (distal - proximal centrosome) | Dif. in anaphase velocity (distal - proximal centrosome) |
|----------------------------------------------------------|----------------------------------------------------------|
|                                                           | The difference in mean velocity between the distal and proximal centrosomes during early anaphase. Positive values indicate a greater velocity for the distal centrosome, etc. |

| Dif. in anaphase displacement along D/P axis (distal - proximal centrosome) | Dif. in anaphase displacement along D/P axis (distal - proximal centrosome) |
|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
|                                                                         | The difference in anaphase displacement along the D/P axis, between the distal and proximal centrosomes. Positive values indicate a greater displacement for the distal centrosome, etc. |

| Dif. anaphase velocity along D/P axis (distal - proximal centrosome) | Dif. anaphase velocity along D/P axis (distal - proximal centrosome) |
|---------------------------------------------------------------------|---------------------------------------------------------------------|
|                                                                     | The difference in mean velocity along the D/P axis between the distal and proximal centrosomes during early anaphase. Positive values indicate a greater velocity for the distal centrosome, etc. |
Supplemental Methods

Worm mounting and live imaging (Figure S1C)

Animals were mounted as described in Methods. Images were acquired on a Quorum WaveFX-X1 spinning disk confocal, controlled by MetaMorph software, using a Leica 63x/1.40-0.60 oil HCX PL APO objective and 50mW 491nm and 568nm diode lasers. Confocal sections of 0.75μm were acquired over 19.5μm for a duration of 40 minutes, using dual camera mode, with ET 525/50 and FF 593/40 emission filters, 200ms exposure time and two Photometrics PRIME BSI CMOS cameras. Sample temperature was regulated using a CherryTemp microfluidic temperature control device (Cherry Biotech; (Velve Casquillas et al., 2011)).

Analysis of mitosis in 1-day old adults (Figure S4)

Raw image data for 1-day old adults were acquired as part of a previous publication (Gerhold et al., 2015) and reanalyzed here. Briefly, synchronized L1 larvae were obtained using a sodium hypochlorite treatment (1.2% NaOCl, 250 mM KOH) and 1-day old adults were imaged 72hr after the L1 stage. Animals were raised at 20°C on either nematode growth medium (NGM) seeded with Escherichia coli strain OP50 or on RNAi feeding plates seeded with the empty L4440 control vector, as described in Methods. Animals were mounted as for L4 larvae and were imaged on a Nikon A1R point scanning confocal microscope (Nikon Canada) using an Apo 40x/1.25 numerical aperture (NA) water-immersion objective and a 488nm Argon gas laser. The strain UM225 (ojls1[unc-119(+)] pie-1::GFP::tbb-2) V which expresses GFP::β-tubulin in the germline was used. CentTracker was used to perform image registration. To maximize our ‘n’, centrosome tracking and pairing were performed using the manual method, as described in Methods. Analysis of spindle orientation relative to the D/P axis was carried out as for L4 larvae with the following modifications: as adult gonads exhibit more curvature and egg laying can deform the tissue during imaging, a single linear D/P axis, as defined for L4 larvae, was not an accurate representation of the tissue axis for all cells. We therefore defined the D/P axis using a ~20μm segment of the gonad centered on the dividing cell during anaphase. The center of the gonad within this region was determined at both ends, using the z-reslice function in ImageJ, and the D/P axis (the vector α, as described in Methods)
was defined as a straight line along the long axis of the ~20μm segment which passed through the gonad center at either end. Distance to the distal tip was measured by summing the length of a series (most frequently 2) line segments from the cell of interest to the distal tip, following the shape of the gonad.

**CentTracker track pair classifier (TPC) construction and validation**

**1.1 TPC algorithm overview**

We applied the random forest algorithm (Breiman, 2001; Louppe, 2015), a well-established machine learning classification technique, for the track pair classification task. In this study, we adopted the random forest implemented by the machine learning platform scikit-learn (Pedregosa et al., 2011), where the final classification is computed by averaging the probabilistic predictions of all tree predictors, as opposed to the majority voting in the original method (Breiman, 2001). The final predicted outcome can be interpreted as the conditional probability of the class given the input.

**1.2 TPC feature construction**

For each putative track pair, we constructed the following eleven features as input to the TPC:

1. The initial spindle length, i.e., the distance (“spindle length”) between two tracks when they first co-appear;
2. The final spindle length, i.e., the distance (“spindle length”) between two tracks when they last co-appear;
3. The maximum spindle length, i.e., the maximum distance between two tracks at any time frame;
4. The minimum spindle length, i.e., the minimum distance between two tracks at any time frame;
5. The track duration, i.e., the duration in which both tracks are present;
6. The congression duration, approximated by the number of continuous time points in which the spindle length is under 4 microns (by default);
7. The center standard deviation, i.e., the standard deviation of the midpoint of two tracks at all time points when the tracks are both present;
8. The normal standard deviation, where the normal is calculated by taking the vector difference between the track i and track j (the order here is determined arbitrarily but remains the same for the given two tracks); 
9. The max intensity, i.e., the maximum of sum of all the values for pixels within the physical radius from the spot center in all time frames;
10. The contrast, defined as:

\[ C = \frac{I_{\text{in}} - I_{\text{out}}}{I_{\text{in}} + I_{\text{out}}} \]

where \( I_{\text{in}} \) is the mean intensity inside the spot volume (using the physical radius), and \( I_{\text{out}} \) is the mean intensity in a ring ranging from its radius to twice its radius.

11. The average estimated diameter of two tracks from the TrackMate output, based on contrast calculation.

After the calculation of the numerical features for all of the putative pairs, we applied max-min normalization to the max intensity and contrast terms, per movie, to adjust for the movie-level intensity differences.

1.3 TPC experimental settings

We followed a standard machine learning routine to train, validate and evaluate the classifier. The data was split into a training (66.6%) set and a test set (33.3%). The test set was kept separate and the training set was used for classifier training and validation. To address the imbalanced class distribution present in the training dataset (false pairs are approximately 9 times more prevalent than true pairs), we applied a stratified 3-fold cross-validation during the hyper-parameter tuning to represent the original class distributions across each train-validation fold. The model with the highest accuracy at the hyper-parameter tuning was selected as the final model. We used three metrics to evaluate the model prediction performance, including accuracy, F1 score and average precision. Accuracy score is defined as:
\[
\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN}
\]

where \(TP\), \(TN\), \(FP\), and \(FN\) stand for true positive, true negative, false positive, false negative, respectively. F1 score is the harmonic mean of the precision \(P\) and recall \(R\), where an F1 score reaches its best value at 1 and worst score at 0, defined as:

\[
F1 = 2 \frac{P \times R}{P + R},
\]

\[
P = \frac{TP}{TP + FP},
\]

\[
R = \frac{TP}{TP + FN}.
\]

The average precision (AP) score summarizes a precision-recall curve as the mean of precisions at each threshold weighted by the change in recall since the last operating point:

\[
AP = \sum \limits_n (R_n - R_{n-1})P_n
\]

where \(P_n\) and \(R_n\) are the precision and recall at the \(n^{th}\) threshold.

We report the model performance on the test data below:

| Class  | Precision | Recall | F1-Score | Support |
|--------|-----------|--------|----------|---------|
| 0      | 0.99      | 1.00   | 0.99     | 3159    |
| 1      | 0.97      | 0.89   | 0.93     | 251     |
| accuracy |          |        |          |         |
| macro avg | 0.98  | 0.94   | 0.99     | 3410    |
| weighted avg | 0.99  | 0.99   | 0.99     | 3410    |

We also trained and evaluated five other classic machine learning classifiers including decision tree, gaussian naive bayes, logistic regression, support vector machine (SVM), and SVM with stochastic gradient descent for baseline methods comparison. While all of the methods tested achieved high accuracy score on the test set (>0.95), the random forest classifier outperforms other methods on both F1 and AP scores by a large margin.
All of the classifiers and evaluation metrics used in this study are implemented with the scikit-learn platform (Pedregosa et al., 2011).

1.5 TPC trainable option
In some cases, users may wish to re-train a TPC specifically tailored to their own dataset which can have distinctly different spindle dynamics and experimental design (e.g., imaging conditions, development stage, etc.). We propose that our framework is highly suitable for this purpose. A user may simply follow the steps detailed in our GitHub walk-through tutorial: https://github.com/yifnzhao/CentTracker, which includes feature construction, filtering, manual validation, hyperparameter tuning, model training, and evaluation. As demonstrated in Figure 3, our framework achieved strong performance in other C. elegans cell types and in cells from other species (here Drosophila).

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